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CRITICAL TESTS OF MISCELLANEOUS ANTHELMINTICS[1]

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INTRODUCTION

By critical tests of anthelmintics is meant tests of these drugs by their administration in definite known doses to animals, with subsequent collection for a suitable length of time of all worms passed and the post-mortem examination of the animals with the collection of all worms then present. Identification of the worms collected ante-mortem and post-mortem, and addition of the numbers of those of the same kind, then give the total number of each kind present at the beginning of the experiment. The relation of the number of each kind passed to the total number present at the beginning of the experiment is ascertained in percentages and this percentage is arbitrarily taken as the percentage of efficacy of the anthelmintic. Such critical testing has been carried on for about 10 years in the Zoological Division of the Bureau of Animal Industry, and hundreds of such experiments show that when properly interpreted the figures thus obtained are a good index of the efficacy of an anthelmintic. Interpretation is necessary, since anthelmintic efficacy varies with several factors such as:

THE DRUG.—Its amount, composition, age, solubility, concomitant effects (such as production of vomition, purgation, or constipation, etc.), mode of administration (such as in hard capsules, soft capsules, enteric-coated capsules, by stomach tube, by rectum, subcutaneously, intramuscularly, intravenously, intratracheally, etc.).

THE OPERATOR.—His accuracy, technic, familiarity with the animal's anatomy, skill in handling animals, etc.

THE EXPERIMENT ANIMAL.—Its special physiology, physical condition, age, peculiar conditions or existing lesions or other pathological conditions, such as atonic and distended stomachs, the complicated stomach of ruminants, etc.

CONCOMITANT PROCEDURE IN TREATMENT.—Fasting, diet, omission of purgatives, use of different purgatives, etc.

THE WORMS.—Number, size, relatively inaccessible locations like the cecum, occurrence in cysts or nodules or in the mucosa or under mucus or hemorrhagic coverings, occurrence of larvae and of adults with protective cuticles, etc.

Like many scientific investigations, these studies consist in correlating two or more variables; the number here is many more than two. Failures in anthelmintic medication may often be explained by careful consideration of these factors; and modifying those factors capable of modification may convert failure into success.

TESTS OF A COMBINATION OF CARBON TETRACHLORIDE, CHENOPODIUM, AND ARECOLINE HYDROBROMIDE FOR WORMS IN DOGS

PROTOCOLS

Dog No. 568; 7 kg.; 2.1 cc. of mixture of 3 parts carbon tetrachloride and 1 part oil of chenopodium by volume, with one-fourth grain of arecoline hydrobromide; animal died within 24 hours; post-mortem, extensive cirrhosis of liver and presence of *Dirofilaria immitis* in the heart; no worms passed and none in digestive tract, hence no conclusions as to efficacy of the treatment. *Dirofilaria*

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immitis is so rare in dogs in the vicinity of Washington, D. C., that probably the few cases found are those of dogs from the southern part of the United States.

Dog No. 548; 6.5 kg.; 1.95 cc. of above mixture with one-eighth grain of arecoline hydrobromide; no worms passed in 4 days; post-mortem, on fourth day, 1 ascarid. Combination entirely ineffective against ascarids.

Dog No. 559; 7 kg.; 2.1 cc. of above mixture with one-fourth grain arecoline hydrobromide; 8 ascarids passed the first day; no worms the next three days; post-mortem, on fourth day, no worms. Combination 100 per cent effective against ascarids.

Dog No. 569; 8.5 kg.; 2.55 cc. of above mixture with one-fourth grain arecoline hydrobromide; no worms passed in four days; post-mortem, on fourth day, 98 whipworms. Combination entirely ineffective against whipworms.

Dog No. 564; 13 kg.; 3.9 cc. of above mixture; first day, 2 whipworms; second day, 25 whipworms; third day, 3 whipworms; fourth day, negative; post-mortem, on fourth day, 32 whipworms. Combination 48 per cent effective against whipworms.

Dog No. 591; 16.5 kg.; 4.95 cc. of above mixture with one-half grain arecoline hydrobromide; first day, 1 hookworm; second and third days negative; fourth day, 112 whipworms; post-mortem, on fourth day, 17 whipworms. Combination 100 per cent effective against hookworms and 87 per cent effective against whipworms.

Dog No. 592; 14.5 kg.; 4.35 cc. of above mixture with one-half grain arecoline hydrobromide; animal appeared sick in a few minutes and died in a few hours. No post-mortem examination and no conclusions.

Dog No. 593; 14.5 kg.; 4.35 cc. of above mixture with one-half grain of arecoline hydrobromide; no worms passed in 4 days; post-mortem, on fourth day, 14 whipworms. Combination entirely ineffective against whipworms.

Dog No. 594; 14 kg.; 4.2 cc. of above mixture with one-half grain of arecoline hydrobromide; first day, 1 hookworm; second, third, and fourth days negative; post-mortem on fourth day negative. Combination 100 per cent effective against hookworms.

Dog No. 595; 6 kg.; 1.8 cc. of above mixture with one-fourth grain arecoline hydrobromide; first day, 2 hookworms; 128 whipworms; second, third, and fourth days negative; post-mortem on fourth day negative. Combination 100 per cent effective against hookworms and whipworms.

Dog No. 596; 13 kg.; 4.2 cc. of above mixture with one-half grain of arecoline hydrobromide; first day, 3 hookworms, 2 whipworms; second day, 1 whipworm; third and fourth days negative; post-mortem, on fourth day, 1 whipworm. Combination 100 per cent effective against hookworms and 75 per cent effective against whipworms.

Dog No. 600; 10 kg.; 3 cc. of above mixture with one-fourth grain of arecoline hydrobromide; first day negative; second day, 2 hookworms; third and fourth days negative; post-mortem, on fourth day, 25 hookworms, 1 whipworm, 6 tapeworms. Combination 7 per cent effective against hookworms and entirely ineffective against whipworms and tapeworms.

Dog No. 601; 13 kg.; 3.9 cc. of above mixture with one-half grain arecoline hydrobromide; first day, 1 hookworm; second, third, and fourth days negative; post-mortem, on fourth day, 19 whipworms. Combination 100 per cent effective against hookworms, entirely ineffective against whipworms.

Dog No. 602; 10 kg.; 3 cc. of above mixture with one-fourth grain of arecoline hydrobromide; first day, 1 hookworm; second, third, and fourth days negative; post-mortem, on fourth day, 18 whipworms. Combination 100 per cent effective against hookworms, entirely ineffective against whipworms.

Dog No. 603; 7 kg.; 2.1 cc. of above mixture with one-fourth grain of arecoline hydrobromide; first day, 6 hookworms; second day. 1 whipworm; third and fourth days negative; post-mortem, on fourth day, 1 whipworm. Combination 100 per cent effective against hookworms and 50 per cent effective against whipworms.

Dog No. 604; 9 kg.; 2.7 cc. of above mixture with one-fourth grain of arecoline hydrobromide; no worms passed in 4 days; post-mortem on fourth day, negative. No conclusions.

Dog No. 605; 5 kg.; 1.5 cc. of above mixture with one-fourth grain of arecoline hydrobromide; first day, 1 ascarid, 5 hookworms; second, third, and fourth days negative; post-mortem, on fourth day, 1 hookworm, 1 whipworm, 64 tapeworms (*Dipylidium* sp.). Combination 100 per cent effective against ascarids, 83 per cent effective against hookworms, entirely ineffective against whipworms and tapeworms.

DISCUSSION

It has been shown experimentally by Hall and Foster (*13*)[2] and by Hall in a number of subsequent papers that chenopodium at the rate of 0.1 cc. per kilogram of weight of animal is a quite dependable anthelmintic for removing ascarids from dogs and that when given with an ounce of castor oil it removes all ascarids present in almost all cases. Chenopodium is also fairly effective in removing hookworms from dogs. It has also been shown by Hall (*18*) and subsequently by Hall and Shillinger (*24*) that carbon tetrachloride at the rate of 0.3 cc. per kilogram of weight of animal is a quite dependable anthelmintic for removing hookworms from dogs, and this has been confirmed in extensive use in veterinary practice for the past 2 or 3 years. The work of Allen (*2*) and of Hanson and Van Volkenburg (*26*) shows that the same is true for hookworms in foxes. Carbon tetrachloride is also fairly effective in in removing ascarids. It has also been shown by Hall and Shillinger (*23*) that arecoline hydrobromide, first proposed as a taeniacide for dogs by Lentz (*32*), removes all tapeworms present in the majority of cases, though it fails to remove some or all in a rather large minority of cases, a thing not uncommon with tapeworm remedies. Theoretically, a combination of carbon tetrachloride, chenopodium, and arecoline hydrobromide should make a good anthelmintic for removing the ascarids, hookworms, and tapeworms from dogs and might prove a useful "shotgun" prescription under conditions preventing fecal examination to determine the sort of worms present. Hall (*18*) found the combination of carbon tetrachloride and chenopodium at a dose rate of 0.3 cc. per kilogram of weight of animal, as given here, entirely effective in removing hookworms and ascarids from dogs. The arecoline hydrobromide should provide the necessary purgation for the chenopodium and carbon tetrachloride.

The fact that the combination removed all the ascarids from 2 of 3 infested dogs, giving what may be termed cures in 67 per cent of cases; failed entirely in one case, or 33 per cent of the cases; and removed 90 per cent of the total of 10 worms present, indicates that there is little loss of efficacy of chenopodium and carbon tetrachloride against ascarids when given with arecoline hydrobromide. That a single ascarid might be missed occasionally is more or less to be expected.

It removed all the hookworms from 7 of 9 infested dogs, giving cures in 78 per cent of the cases; removed 83 per cent of the worms in one case and 7 per cent in another, giving partial cures in 22 per cent of the cases; and removed 46 per cent of the total of 48 worms present. These facts indicate that carbon tetrachloride and chenopodium, when given with arecoline hydrobromide, show a distinct loss of efficacy and dependability against hookworms.

It removed all the whipworms from 1 of 11 infested dogs, giving cures in 9 per cent of the cases; removed no worms in 6 cases, or 55 per cent of the cases; removed 87, 75, 50, and 48 per cent of the worms in 4 cases, giving partial cures in 36 per cent of the cases; and removed 57 per cent of the total of 476 worms present. This is not a bad showing, and is perhaps better than that of the individual drugs used in the combination, although this is not easy to ascertain owing to the erratic action of drugs against whipworms.

Whipworms are not good subjects for ordinary anthelmintic tests, as the efficacy of a drug appears to depend on the accident of the entry of the drug into the cecum, rather than on the effect of the drug on the worms with which it comes in contact.

It removed none of the 70 tapeworms present in two cases, giving complete failures with these worms. The striking thing about the experiments is this total failure of the arecoline hydrobromide against tapeworms. The authors have shown in a previous paper (*23*) that all the tapeworms were removed from 4 of 7 animals treated and none from 3 animals, so it is evident that dependable action can not be expected in nearly all cases. Unfortunately, the series of available animals included too few with tapeworms to make this a good test, but it certainly gives little reason for expecting much in the way of tapeworm removal from this combination.

In general, this combination does not give in test the results that would be expected from a theoretical consideration. It would probably maintain a rather high efficacy against ascarids, show a decided decrease of efficacy against hookworms and tapeworms, and at least average efficacy against whipworms, as compared with its constituents, but could hardly be recommended as a useful combination for cases where fecal examinations

[2] Reference is made by number (italic) to "Literature cited," pp. 331-332.

were not made to ascertain the kinds of worms present. It would appear to be better practice in such cases to give the carbon tetrachloride or the mixture of carbon tetrachloride and chenopodium for ascarids and hookworms and such whipworms as might incidentally be removed, and a week or two later to give arecoline hydrobromide.

The deaths of 2 animals out of 17 within 24 hours after treatment can not be definitely correlated with the administration of the drugs as the sole factor or even the primary factor, but it suggests that the treatment is none too safe and that in routine practice it would be injurious to an unduly high percentage of animals treated.

TESTS OF BENZYL-PHENOL FOR REMOVING WORMS FROM DOGS

PROTOCOLS

Dog No. 606; 8 kg; 20 grains in hard capsules; first day negative; second day, 3 whipworms; third and fourth days, negative; post-mortem, on fourth day, 61 whipworms, 11 hookworms, 3 tapeworms. Benzyl-phenol about 5 per cent effective against whipworms; entirely ineffective against hookworms and tapeworms.

Dog No. 607; 12 kg.; 20 grains one day and 30 grains the following day, given in hard capsules; no worms in 3 days after first dose; post-mortem, on third day, 5 whipworms, 3 hookworms, and 3 tapeworms. Wholly ineffective against whipworms, hookworms, and tapeworms.

Dog. No. 611; 14.5 kg.; 30 grains in hard capsules; first day, 3 hookworms; second, third and fourth days negative; post-mortem, fourth day, 9 hookworms, 6 whipworms. Effective 25 per cent against hookworms; entirely ineffective against whipworms.

Dog No. 608; 6.5 kg.; 20 grains in hard capsules; no worms in 4 days; post-mortem, on fourth day, 15 hookworms, 15 whipworms, and 3 tapeworms. Entirely ineffective against hookworms, whipworms, and tapeworms.

Benzyl-phenol in doses of 20 and 30 grains in single dose and in doses of 20 grains followed by 30 grains the following day removed three of 41 hookworms, or 7 per cent; 3 of 90 whipworms, or 3 per cent; and 1 of 9 tapeworms.

DISCUSSION

The anthelmintic value of phenols has been investigated to some extent in this country and abroad, and various phenols, such as coal-tar creosote, have been used empirically for years.

Stiles (*41*) reported that coal-tar creosote (the phenol content not given) would kill stomach worms in sheep in some cases at least and gave good clinical results in sheep, calves and grown cattle in a number of cases.

Hall and Foster (*13*) tested certain coal-tar creosote preparations on four sheep, with unsatisfactory results. Two sheep died the day after treatment and only four nodular worms were recovered from the manure of all animals. The post-mortem findings were inconclusive, but failed to show the treatments as of value. Hall and Foster also tested a coal-tar creosote preparation on two dogs. One dog passed 2 ascarids and on post-mortem examination had 3 ascarids and 3 hookworms. The other dog passed a tapeworm without the head, but as the dog had ascarids, hookworms and tapeworms, killing the animal for post-mortem examination did not seem worth while.

Caius and Mhaskar (*6*) reported tests of propenyl phenols on hookworms in man, from which they concluded that these phenols have well-marked anthelmintic properties which are not associated with the unsaturated side chain but with the phenol group. In other papers they state that the efficacy of thymol and betanaphthol is correlated with the free phenolic-hydroxyl group in these compounds.

In this connection it may be recalled that the phenols are a group of organic compounds composed of hydroxy derivatives of the benzene series, the hydroxyl radical being linked directly to the nucleus. The refined phenols include phenol, cresol and the higher phenols. In benzyl-phenol, benzoic acid, which is also a benzene ring derivative, is linked to the benzene nucleus of phenol, leaving the free phenolic hydroxyl group present as in thymol and betanaphthol. The protocols of the present investigation show that in spite of the presence of this phenolic hydroxyl group, benzyl-phenol has only about 5 per cent efficacy against hookworms, which may be regarded as the only suitable test worms present in these animals. With the doses used, a moderate amount of inflammation was present in the small intestine of three animals, and an extensive serous infiltration in the lower part of the small intestine and the cecum of the other. Dr. A. R. Albright states that benzyl phenol is

less toxic and less irritant to the mucosa than thymol and is better tolerated in the stomach. Such facts would favor this substance for use in place of thymol provided the anthelmintic efficacy were as high as that of thymol or higher. In experiments reported by Hall and Foster (*13*), thymol in doses of 4.5 to 26 grains administered in single and repeated doses to 9 animals removed 23 of 151 hookworms, or 15 per cent, the maximum efficacy, 50 per cent, being with doses of about 2 grains per kilogram (or 20 grains for an average-sized dog) repeated three times. With these repeated treatments, 1 dog passed 89 per cent of its hookworms and the others passed none. Apparently neither thymol nor benzyl-phenol is very effective against hookworms in dogs, although the doses used, approximately 2 grains per kilogram, or 1 grain per pound, are those recommended for thymol for removing hookworms from dogs. Both of these drugs appear much inferior to carbon tetrachloride for this purpose.

TESTS OF ETHYLENE DICHLORIDE FOR WORMS IN DOGS

PROTOCOLS

Dog No. 617; 9.5 kg.; 2.85 cc. in hard capsules; first day, 2 hookworms; no worms the next 3 days; post-mortem, on fourth day, 21 hookworms, 47 whipworms, 94 tapeworms. Drug 9 per cent effective against hookworms; entirely ineffective against whipworms and tapeworms.

Dog No. 645; 7 kg.; 3.75 cc. in hard capsules; no worms in 4 days; post-mortem, on fourth day, 3 hookworms, 6 whipworms, 5 tapeworms. Drug entirely ineffective against hookworms, whipworms, and tapeworms.

Dog No. 638; 14 kg.; 7 cc. by stomach tube; 1 hookworm and 1 whipworm the second day; no other worms in 4 days; same amount then given in hard capsules; first day, 11 hookworms; second day, 2 whipworms; no worms the next 2 days; post-mortem, on fourth day after second treatment, 6 hookworms, 91 whipworms, 4 tapeworms. Two treatments 67 per cent effective against hookworms, 3 per cent effective against whipworms, entirely ineffective against tapeworms.

DISCUSSION

Chloroform has been more or less used in human medicine against hookworms and has been especially recommended by Alessandrini (*1*). Schultz (*39*) found it effective in removing hookworms from dogs. Hall and Foster (*13*) found it more effective in single dose for removing hookworms than other drugs tested by them and found its combination with chenopodium quite effective, as did Hall (*16*) in later experiments. The findings with chloroform, $CHCl_3$, led Hall (*18*) to test carbon tetrachloride, CCl_4, against hookworms, this drug being found more effective against hookworm in dogs than any other drug yet known. It is of interest in this connection to note that Caius and Mhaskar (*4*) found chloroform very effective in removing hookworms from man, and a year later (*5*) stated in connection with the efficacy of this drug: "This raises the question whether a narcotic, less toxic to the host than chloroform, assisted by a purgative, would not prove a very efficient remedy for the removal of hookworms." The prophecy was fulfilled in the discovery of the value of carbon tetrachloride, Hall's first paper (*18*) on this appearing the same month (April) as that of Caius and Mhaskar. Caius and Mhaskar later carried on tests of carbon tetrachloride, confirming its value against hookworms in man, and in a summary (*7*) they state that the value of carbon tetrachloride is correlated with the cumulative effect of the halogen atoms. On this basis ethylene dichloride, $C_2H_4Cl_2$, should rank somewhere below chloroform as an anthelmintic. In the present experiments this chemical in single doses of 0.3 to 0.5 cc. per kilogram removed from 0 to 9 per cent of the hookworms; and in two doses of 0.5 cc. per kilogram removed 67 per cent. These findings apparently sustain the theoretical likelihood that this ethane derivative with two atoms of chlorine is less effective than the methane derivative, chloroform, with three atoms, and this in turn is less effective than the methane derivative, carbon tetrachloride, with 4 atoms of chlorine. The presence of hydrogen seems to increase the solubility of the chemical, carbon tetrachloride being soluble 1 part in 1,250 of water and chloroform 1 part in 161 of water; the solubility of ethylene dichloride is unknown to us but is probably closer to that of chloroform than to that of carbon tetrachloride. With the increased solubility there is an increase in undesirable systemic effects on the host animal, and a diminished efficacy against worm parasites. It may be concluded that ethylene dichloride shows a slight efficacy against hookworms in dogs, as would be expected from its chemical composition; but

this efficacy is distinctly less than that of carbon tetrachloride, as would also be expected since the halogen concentration, to which the anthelmintic efficacy is apparently due, is distinctly less in ethylene dichloride than in carbon tetrachloride.

TESTS OF FERROUS SULPHATE FOR REMOVING WHIPWORMS FROM DOGS

PROTOCOLS

Dog. No. 585; 8.5 kg.; 56.4 grains in 30 cc. of water with 5 grains of calomel; no worms passed in 5 days; post-mortem on fifth day, 2 whipworms, 1 hookworm, 3 tapeworms. Entirely ineffective.

Dog No. 586; 8.5 kg.; 42.3 grains in 22.5 cc. of water; no worms in 5 days; post-mortem, on fifth day, 13 whipworms. Entirely ineffective.

Dog No. 587; 11 kg.; 28.2 grains in 15 cc. of water with 5 grains of calomel; no worms in 5 days; post-mortem, on fifth day, 24 whipworms, 2 hookworms, 4 tapeworms. Entirely ineffective.

Dog No. 588; 11 kg.; 32.9 grains in 17.5 cc. of water; no worms in 5 days; post-mortem, on fifth day, 4 whipworms. Entirely ineffective.

Dog No. 589; 13 kg.; 87.8 grains in 45 cc. of water with 5 grains of calomel; first day, 1 whipworm; second day, 2 whipworms; third day, 1 whipworm; fourth and fifth days, negative; post-mortem, on fifth day, 109 whipworms. Treatment 4 per cent effective against whipworms.

Dog No. 590; 12.5 kg.; 70.5 grains in 37.5 cc. of water; no worms in 5 days; post-mortem, on fifth day, 89 whipworms and 3 tapeworms. Entirely ineffective.

DISCUSSION

It has been pointed out by Hall (*10; 12*) that even feeble anthelmintics, such as ferrous sulphate, will occasionally remove whipworms from dogs; but, as noted above (p. 315), the efficacy of a drug in removing these worms depends on its entry into the cecum. This entry might be insured by the use of a drug of low toxicity and little irritant quality that could be given in repeated doses or in massive doses, as previously noted by the authors and as stated by Lambert (*29*). For repeated doses santonin seems the most satisfactory drug known at present. For bulky doses the latex of *Ficus laurifolia*, a fig which grows in South and Central America, appears to be favored in human medicine. This has been used by physicians in the region in which it grows, and has been recommended by Berrio, Mouatt-Biggs, and others. It is given in the comparatively large doses of 10 to 45 gm., and is said to be the best drug known for administration in this way against whipworms.

Theoretically, ferrouss ulphate might prove to be of value in this connection, since its toxicity is low and its slow movement through the digestive tract might insure its entry into the cecum in a fair number of cases when given in rather large doses. The drug is often prescribed in veterinary medicine for worms in various animals, although its use is almost entirely on an empirical basis. The present experiments indicate that in doses of 28.2 to 87.7 grains to dogs weighing from 8.5 to 13 kg., it shows little efficacy against whipworms and can not be depended on to remove these worms in single doses.

TESTS OF CHENOPODIUM INTRAMUSCULARLY AND INTRAVENOUSLY FOR REMOVING WHIPWORMS FROM DOGS

PROTOCOLS

Dog No. 549; 6 kg.; 0.5 cc. chenopodium intramuscularly in large muscles of hind leg; no worms in 4 days. Treatment ineffective, as this animal showed whipworm eggs in feces.

Dog No. 552; 9 kg.; 1 cc. chenopodium intramuscularly in large muscles of hind leg; no worms in 4 days. Treatment ineffective, as this animal showed whipworm eggs in feces.

Dog No. 609; 8 kg.; 1 cc. chenopodium intravenously; no worms in 2 days; animal dead second day; post-mortem showed 6 whipworms attached in cecum, 1 whipworm unattached in colon, 12 tapeworms (*Dipylidium* sp.). Treatment comparatively ineffective, assuming, as seems probable, that the whipworm unattached in the colon would have passed out in a day or so and that the treatment should probably be credited with its removal.

Dog No. 610; 7 kg.; 0.5 cc. intravenously; 1 whipworm the second day; some tapeworm fragments, but no heads, during 4 days; post-mortem, on fourth day, 14 whipworms, 5 hookworms, and 28 tapeworms. Drug 7 per cent effective against whipworms; entirely ineffective against hookworms and tapeworms.

DISCUSSION

The idea of removing whipworms by means of intramuscular injections seems to have occurred first to Strong (*42*), who administered to human patients thymol and chenopodium, each in sterile olive oil, without results in the form of whipworms passed or noticeable diminution in the number of eggs present in the feces.

More recently Lambert (*29*) has reported tests on human patients of intramuscular and intravenous injections of chenopodium. For his intramuscular injections Lambert used in two cases a mixture of 60 cc. oil of chenopodium, 60 cc. of camphorated oil, and 4 grams of resorcin, giving 4 cc. in one case and 10 cc. in another. After the dose of 4 cc. (equivalent to 2 cc. of chenopodium), the patient passed 4 hookworms in the course of 4 days; an anthelmintic by mouth removed 89 hookworms, 1 ascarid, and 1 whipworm. After the dose of 10 cc. (equivalent to 5 cc. of chenopodium), the patient passed 2 hookworms and 1 ascarid in the course of 3 days; an anthelmintic by mouth removed 155 hookworms and 1 ascarid. After a dose of 3 cc. of undiluted chenopodium the patient passed 3 hookworms, 22 whipworms, 9 pinworms; no anthelmintic was given by mouth, but hookworm eggs persisted in the feces after treatment.

For intravenous injections Lambert used pure oil of chenopodium in doses of 2 cc. in one case and 1.5 cc. in two other cases. After the dose of 2 cc. the patient passed 11 whipworms and 882 pinworms in the course of 3 days; chenopodium given by mouth removed 33 hookworms and 1 whipworm. One patient given 1.5 cc. intravenously passed 19 whipworms and 2 ascarids in the course of 4 days; no anthelmintic was given by mouth, but fecal examination showed hookworm eggs present in the feces. The other patient given 1.5 cc. intravenously passed 30 whipworms and 2 ascarids in the course of 3 days; no anthelmintic was given by mouth, but fecal examination showed hookworm eggs present in the feces.

From Lambert's experiments it appears that chenopodium intramuscularly will remove some whipworms, ascarids, hookworms, and pinworms, being apparently rather effective against whipworms in some cases, but not promising anything vaulable against the other worms named. Intravenously chenopodium removed whipworms, ascarids, and pinworms, the efficacy against whipworms being most evident, with the possibility that this treatment is fairly effective against ascarids and pinworms, although the evidence is not sufficient to warrant the idea that it is certainly as effective as other measures known to be less dangerous.

In the present experiments intramuscular injections of chenopodium made the animals lame and failed to remove whipworms. It does not therefore appear to be a promising mode of treatment for removing whipworms from dogs. Intravenous injections apparently removed 1 whipworm from each of 2 dogs, leaving 6 and 14 worms, respectively. Judging from Lambert's results a much higher efficacy might be obtained in individual cases, but the results in these two cases do not look promising, although intravenous injections show a greater efficacy in removing whipworms than intramuscular injections show.

A low efficacy seems indicated in the case of dogs and a high efficacy in human patients, judging from the usual numbers present in man in the region involved. This method of treatment should be investigated further. Possibly drugs more satisfactory than chenopodium could be found for intravencus injection. In the human cases syncope followed the injections; and in one dog the syncope immediately after the injections was followed by general incoordination, pronounced sickness with decubitus, and death the second day.

TESTS OF NOVARSENOBENZOL IN SOLUTION BY MOUTH, SUBCUTANEOUSLY AND INTRAVENOUSLY FOR WHIPWORMS IN DOGS

PROTOCOLS

Dog No. 612; 10.5 kg.; 0.6 gm. subcutaneously; no worms in 6 days; the dog died after 11 days; too badly decomposed to warrant post-mortem examination following negative results.

Dog No. 613; 5 kg.; 0.15 gm. intravenously; no worms in 2 days; 0.75 gm. intravenously the third day; no worms in 4 days; post-mortem, on fourth day after second treatment, 19 whipworms, 9 tapeworms. Wholly ineffective.

Dog No. 615; 15 kg.; 0.45 gm. intravenously; no worms in 4 days; no post-mortem. Apparently wholly ineffective.

Dog No. 616; 13 kg.; 0.3 gm. by mouth; no worms in 4 days; after 42 days this dog (now weighing 18.5 kg.)

was given 0.6 gm. by mouth; first and second days, no worms; third day, 4 whipworms; fourth day negative; 0.6 gm. by mouth on fourth; fifth day, 2 whipworms; sixth day, 3 whipworms; seventh day negative; eighth day, 1 whipworm; 0.6 gm. by mouth on eighth day; ninth and tenth days negative; eleventh day, 1 whipworm; twelfth day, 1 whipworm; 0.6 gm. by mouth on twelfth day; fecal examination negative from thirteenth to twenty-sixth day; doses of 0.6 gm. by mouth on sixteenth, twentieth, and twenty-fifth days; post-mortem, twenty-sixth day, 9 ascarids, 14 hookworms, 25 tapeworms (*Dipylidium* sp.). Treatment 100 per cent effective against whipworms, ineffective against hookworms, ascarids, and tapeworms.

DISCUSSION

Lundsgaard (*33*) has reported that 12 days after the last of five doses of 0.3 to 0.9 gm. of neosalvarsan a human patient passed a decomposed strobila of *Taenia saginata*. The present experiments were too brief to determine whether novarsenobenzol might similarly affect dog tapeworms. They do not indicate that novarsenobenzol in single doses subcutaneously or intravenously as given is of value in removing whipworms or other worms from dogs. Theoretically, the whipworm, which has its head and anterior body usually embedded in the mucosa of the cecum, or occasionally in that of the colon, might be poisoned in feeding on blood or serum. The action of chenopodium indicates that something of the sort is possible, but apparently novarsenobenzol is not a suitable drug for the purpose.

Given by mouth in repeated doses, novarsenobenzol removed all whipworms, a total of 12, after four doses amounting to 2.1 gm., no worms being present at the time three subsequent doses for a total of 1.8 gm. were given. So far as efficacy in repeated doses is concerned, this drug is apparently capable of replacing santonin in removing whipworms. Its efficacy by mouth is apparently along the lines we have previously outlined—that whipworms are easily removable by even feeble anthelmintics provided the anthelmintic comes in contact with the worms—and such contact can be insured by the use of repeated doses of a drug of relatively low toxicity and little irritant properties, or of large doses of such a drug in cases where large doses may be used. As regards its safety, the dog dosed by mouth showed a somewhat darkened liver and some inflammation of the small intestine after a total of 3.9 gm. administered; the findings do not indicate that the drug as given is dangerous, although little can be concluded from the findings on one animal. The principal objection to its use would be its expense.

TESTS OF TARTAR EMETIC IN SOLUTION INTRAVENOUSLY FOR WHIPWORMS IN DOGS

PROTOCOLS

Dog No. 637; 6.5 kg.; 1 grain in 1 cc. of sterile distilled water; no worms on first day; animal died in 24 hours; post-mortem showed 5 whipworms, 112 tapeworms (*Dipylidium* sp.). Entirely ineffective against whipworms and tapeworms. This dog showed extensive hemorrhages of the stomach and small intestine. The liver was yellow and friable and on section by Dr. Leigh Giltner of the Pathological Division of this bureau showed passive congestion with capillary hemorrhage, together with some atrophy of the cells, perhaps resulting from pressure of hyperemia.

Dog No. 634; 6.5 kg.; 1 grain in 1 cc. of distilled water; no worms in 4 days; no post-mortem at this time owing to total inefficacy of treatment (dog showed whipworm infestation on fecal examination).

Dog No. 633; 12 kg.; 0.5 grain in 1 cc. sterile distilled water; no worms in 4 days; no post-mortem at this time owing to total inefficacy of treatment (dog showed whipworm infestation on fecal examination).

DISCUSSION

Tartar emetic is well established in human medicine as a drug which is effective against blood flukes when administered intravenously. Theoretically it might be valuable against whipworms. The present tests indicate that in single doses of 0.5 to 1 grain it is not effective, and in certain susceptible dogs it may be highly toxic in doses of 1 grain.

TESTS OF A PROPRIETARY DRUG FOR TAPEWORMS IN DOGS

PROTOCOL

Dog No. 614; 12 kg.; 3.3 cc. (a dose rate of 2 minims per pound); first day, 6 tapeworms; next 3 days negative;

post-mortem, on fourth day, 62 tapeworms, 12 hookworms, 2 whipworms. Drug 9 per cent effective against tapeworms, entirely ineffective against hookworms and whipworms.

DISCUSSION

Since discoverers of especially effective drugs have the alternative of publishing their findings for general use or marketing their products for profit, there is always the possibility that proprietary remedies may at times be found more effective than the drugs generally known and used. For this reason the writers occasionally test proprietary anthelmintics. The proprietary preparation under consideration here is advertised as a safe and effective anthelmintic for tapeworms in dogs. A test indicates that it may fail to remove more than 9 per cent of the tapeworms from a dog. This is not surprising, as drugs intended to remove tapeworms do not show in a general way the dependable efficacy of the best drugs for the removal of nematodes. Thus occasional failures to get tapeworms occur with such standard taeniacides as male fern, kamala, areca nut, arecoline hydrobromide, etc. The proprietary in question is probably effective in many cases.

CHENOPODIUM AND MAGNESIUM SULPHATE, SIMULTANEOUSLY ADMINISTERED, TESTED FOR TOXIC EFFECTS AND EFFECTS ON WORMS IN DOGS

PROTOCOLS

Dog No. 615; 16 kg.; chenopodium at the lethal dose rate, 0.6 cc. per kilogram (9.6 cc.), by stomach tube, followed immediately by 90 cc. of a saturated solution of magnesium sulphate; fecal examination negative and no worms passed in 4 days; the animal continued in good health and was not killed; animal negative on preliminary fecal examination.

Dog No. 634; 8 kg.; chenopodium at the lethal dose rate, 0.6 cc. per kilogram (4.8 cc.), by stomach tube, followed immediately by 60 cc. (2 ounces) of a 50 per cent concentration of magnesium sulphate solution; fecal examination negative and no worms passed in 4 days; post-mortem examination on fourth day negative for worms; the only lesion was a slight inflammation in places in the small intestine.

Dog No. 635; 11 kg.; chenopodium at the lethal dose rate, 0.6 cc. per kilogram (6.6 cc.), by stomach tube, followed immediately by 60 cc. (2 ounces) of a 50 per cent concentration of magnesium sulphate solution; dog vomited in a few minutes and died in about 12 hours; no worms passed; post-mortem showed 5 whipworms. This dog's stomach had slightly inflamed areas; the small intestine showed excessive catarrhal enteritis, and had a marked odor of chenopodium; the cecum and large intestine were normal in appearance, though there was catarrhal material in the lumen of the large intestine, possibly from the small intestine; the lungs were much congested.

Dog No. 650; 8 kg.; chenopodium in double the minimum lethal dose rate of 0.5 cc. per kilogram, or 1 cc. per kilogram (8 cc.), by stomach tube, followed immediately by 60 cc. (2 ounces) of a 50 per cent concentration of magnesium sulphate solution; fecal examination negative, no worms passed in 4 days, none present post-mortem on fourth day; all organs apparently normal post-mortem.

Dog No. 651; 11 kg.; chenopodium at the therapeutic dose rate for ascarids, or 0.1 cc. per kilogram (1.1 cc.), by stomach tube, followed immediately by 60 cc. (2 ounces) of a 50 per cent concentration of magnesium sulphate solution; 1 ascarid the first day; no worms the next 3 days; post-mortem, on fourth day, 1 ascarid. Efficacy against ascarids, 50 per cent.

Dog No. 636; 8 kg.; chenopodium at the therapeutic dose rate for ascarids, or 0.1 cc. per kilogram (0.8 cc.), by stomach tube, followed immediately by 60 cc. (2 ounces) of a 50 per cent concentration of magnesium sulphate solution; 14 ascarids the first day; no worms the next 3 days; post-mortem, on fourth day, 3 whipworms, 4 tapeworms. Treatment 100 per cent effective against ascarids; entirely ineffective against whipworms and tapeworms.

Dog No. 648; 7 kg.; chenopodium at the therapeutic dose rate for ascarids, or 0.1 cc. per kilogram (0.7 cc.), by stomach tube, followed immediately by 60 cc. (2 ounces) of a 50 per cent concentration of magnesium sulphate solution; 1 hookworm the second day; no worms the first, third and fourth days; post-mortem, on fourth day, 54 whipworms. Treatment 100 per cent effective against hookworms; entirely ineffective against whipworms.

Dog 636 was killed by the intraperitoneal injection of 150 cc. of a saturated solution of magnesium sulphate; the animal lay down, became unconscious, and died in about 10 minutes. The

post-mortem lesions due to the drug consisted in pronounced vascular injection of the abdominal blood vessels. Dog 648 was killed by the intraperitoneal injection of 20 cc. of a saturated solution of magnesium sulphate; the animal lay down, became unconscious, and died in about 15 minutes. The lesions due to the injection were similar to those in the case of dog No. 636. This method of killing dogs has been recommended, but no previous report of the post-mortem lesions has been noted.

DISCUSSION

Macht and Finesilver (*34*) have pointed out that the simultaneous administration of magnesium sulphate with other drugs diminishes or prevents the absorption of the other drugs, apparently by virtue of the well-known salt action which causes a flow of fluid from the walls of the digestive tract to the lumen and thus inhibits an absorption current in the opposite direction. It follows from their conclusions that magnesium sulphate should not be given with other drugs where absorption of these drugs with consequent systemic effects is desired. However, it would also appear that in the case of anthelmintics, and possibly of intestinal antiseptics, the simultaneous administration of magnesium sulphate would be valuable, all other things being equal, since such substances are commonly more or less toxic, their systemic effects are not desired, and it is advisable to limit their action to that on the fauna and flora of the digestive tract if possible, leaving the host relatively unaffected. The present experiments show that magnesium sulphate saved the lives of two dogs given 0.6 cc. per kilogram of chenopodium, a little more than the minimum lethal dose of 0.5 cc. per kilogram, and of one dog given double the minimum lethal dose, but failed to save one dog given 0.6 cc. per kilogram.

The question arises whether the simultaneous administration of salts with anthelmintics will lessen the efficacy of the anthelmintic. The experiments reported above show that in therapeutic doses chenopodium with magnesium sulphate removed 15 out of 16 ascarids from two infested dogs, all the hookworms (*1*) from another, and failed to remove tapeworms and whipworms as might be expected. This drug therefore maintains its anthelmintic efficacy when given simultaneously with magnesium sulphate. Hall and Shillinger (*25*) have reported tests of the effect of magnesium sulphate administered simultaneously with carbon tetrachloride, and find the latter as effective against hookworms when administered in this manner as when administered without the salts. They were unable to obtain definite results as to the diminished toxicity of carbon tetrachloride given with the salts, as dogs tolerated very large doses of carbon tetrachloride when given alone or with the salts. In human practice Lambert (*28*) finds that carbon tetrachloride is as effective when given with the salts as when given alone, and that there is less complaint of headache and dizziness after its administration in this manner. This indicates that there is an increase in the safety of carbon tetrachloride administration when given with salts.

The additional safety of anthelmintics when given with magnesium sulphate must be checked against another factor than inhibition of absorption by virtue of the osmotic action occurring in the presence of salts. This is the factor of purgation itself. Hall (*12*) has noted that, contrary to a frequently repeated statement, it is not dangerous to give male fern with castor oil. He cites experiments in which dogs given lethal doses of male fern with adequate doses of castor oil survived, whereas the same dose without the castor oil was fatal. In human medicine various physicians, such as Leichtenstern (*31*), concur in this. Hall also reports an experiment in which the lethal dose of male fern given with 6 grains of calomel was not fatal to a dog. In the same paper he reports that dogs survived the administration of dangerously high to lethal doses of chenopodium with large doses of castor oil. Thus purgation alone by means of such purgatives as castor oil or calomel is highly protective against anthelmintics, as clinical experience also indicates, and may of itself save an animal from an otherwise lethal dose of an anthelmintic. On theoretical grounds, sustained by experimental evidence, the salt action of magnesium sulphate should add to this purgative action the inhibition of absorption by virtue of pronounced osmosis with fluids entering the lumen of the digestive tract. Even with this added action, magnesium sulphate, as one of the present experiments shows, may fail occasionally to save one animal from a lethal dose, although saving another animal from a dose at a higher rate.

The further element of speed of action must be considered in selecting purgatives in connection with anthelmintics. There has been more or less

debate on the value of castor oil and magnesium sulphate in connection with chenopodium. So far as evidence is available, the results are very good when castor oil is given at the same time as chenopodium. When purgatives are not administered until 2 or 3 hours after a final dose of chenopodium, magnesium sulphate is preferable to castor oil, largely because its more rapid action is needed when a depressant drug like chenopodium has been in the digestive tract for several hours and has established a condition of stasis and constipation. Yet the action of magnesium sulphate, other than its mere rapidity in causing purgation, indicates that it is preferable to castor oil even for simultaneous administration with anthelmintics, provided no diminution of anthelmintic efficacy follows. It has been shown, as noted above, that no diminution occurs with castor oil, and the same is apparently true in regard to magnesium sulphate, in connection with chenopodium.

TESTS OF CARBON TETRACHLORIDE AND MAGNESIUM SULPHATE, SIMULTANEOUSLY ADMINISTERED, ON WORMS IN MONKEYS

A male chimpanzee, about 1.5 years old was given 3 cc. of carbon tetrachloride with an ounce of magnesium sulphate dissolved in 90 cc. of water, by stomach tube; it passed 2 hookworms and 14 nodular worms the first day; 3 nodular worms the second day; a total of 2 hookworms and 17 nodular worms.

A female chimpanzee, about 1.5 years old, given same treatment as above, passed 1 hookworm the first day, 1 whipworm the second day; a total of 1 hookworm and 1 whipworm.

These animals were treated at the request of the owner and were not killed. Although the actual efficacy of the drug as used can not be given, the experiment is recorded here since it indicates that carbon tetrachloride as given will remove hookworms and nodular worms from monkeys. There is so little information in regard to anthelmintics for use in these animals that even this item might prove useful. Toxicity tests of carbon tetrachloride on monkeys were reported by Hall (*19*), Lake (*27*) and Hall and Shillinger (*24*). Their reports show that monkeys tolerate single doses of 6 cc. per kilogram, and doses of 1 to 5 cc. for animals weighing 2.21 to 2.63 kg. repeated 12 to 16 times for totals of 16 to 66 cc. The drug removed at least 1 whipworm and a number of heterakids (*Subulura distans*), but since the animals were not killed the exact efficacy was not ascertained.

TESTS OF THE EFFICACY AND SAFETY OF CARBON TETRACHLORIDE WHEN ADMINISTERED SIMULTANEOUSLY WITH MAGNESIUM SULPHATE TO SHEEP

PROTOCOLS

Sheep No. 6c; 10 cc. carbon tetrachloride in hard capsules followed immediately by approximately 128 gm. (4 ounces) of magnesium sulphate in 250 cc. of water; first day, 96 stomach worms (*Haemonchus contortus*), 102 small trichostrongyles, 5 tapeworms with many tapeworm fragments; second day, 94 stomach worms, 72 small trichostrongyles, 1 nodular worm, a few tapeworm fragments; third day negative; fourth day, 2 stomach worms; post-mortem, on fourth day, 1 young female stomach worm embedded in coagulum, 1 whipworm, 10 tapeworms (one tapeworm mature, the others either very young or the remainders of strobila with the major posterior portions removed by the treatment). Since the coagulum in which the young stomach worm was embedded (the regular method of occurrence of young forms) presumably protected it against anthelmintic action, the treatment may be considered 100 per cent effective against stomach worms. Hall and Shillinger (*24*) have pointed out that most of the worms killed in the stomach by anthelmintics become just so much proteid material and are digested, so that probably only the worms near the pylorus escape digestion and appear in the manure as dead worms or worm fragments. The efficacy of anthelmintics against such worms is therefore higher than that indicated by a comparison of the number of worms passed and the number present post-mortem. The stomach worms present in the check animal, No. 9c, noted below, is evidence of the high efficacy of the treatment in this case and the following cases. The treatment was also 100 per cent effective in removing small trichostrongyles, indicating that the high efficacy noted in some cases in the writers' previous report is increased by the simultaneous administration of the magnesium sulphate. The efficacy against nodular worms, of which only one was found, is also 100 per cent; experience shows that it is substantially as difficult to secure 100 per cent efficacy

when only one worm is present, or a few worms, as when many are present. The efficacy against tapeworms, 33 per cent, is high for carbon tetrachloride in case of tapeworms, and suggests that the use of the magnesium sulphate has increased the efficacy of the drug against these worms. The treatment failed to remove the whipworm present. The explanation for such failures is given in the discussion of whipworms in dogs.

Sheep No. 7c; 10 cc. carbon tetrachloride in hard capsules followed immediately by approximately 128 gm. (4 ounces) of magnesium sulphate in 250 cc. of water; no worms passed in 4 days; post-mortem, on fourth day, a fragment of a dead stomach worm in stomach, 10 whipworms. The treatment was 100 per cent effective against stomach worms; the dead worm and the presence of stomach-worm eggs in the feces before treatment, together with the number of stomach worms present in the check animal, No. 9c, show that these worms were present and that all were killed and digested, no worms appearing in the feces. If small trichostrongyles were originally present they were destroyed and passed in unrecognizable condition. The treatment failed to remove whipworms.

Sheep No. 8c; 10 cc. carbon tetrachloride in hard capsules followed immediately by approximately 128 gm. (4 ounces) of magnesium sulphate in 250 cc. of water; first day, 6 nodular worms; no worms the next 3 days; post-mortem, on fourth day, negative. The treatment was entirely effective against nodular worms, and apparently entirely effective against stomach worms, as eggs of these worms were present before treatment.

Sheep No. 9c, a check animal, not treated, was examined post-mortem to determine the probable stomach-worm infestation present in the other animals before treatment. This sheep had 1,434 stomach worms, and it is probable that somewhat similar infestations were present in the three animals treated, the drug destroying all the worms present except the young one still embedded in coagulum.

DISCUSSION

Hall and Shillinger (*24*) found that doses of 8, 12, 15, 18, 24, and 48 cc. carbon tetrachloride left no stomach worms in eight sheep infested with these worms as shown by fecal examination, a check animal having 612 stomach worms. A dose of 4 cc. left 14 stomach worms, indicating that this dose was too small to remove all of the worms, although a very high efficacy, perhaps 98 per cent, is probably indicated by the findings on the check animal and what is known of the digestion of these worms when dead in the stomach, coupled with the efficacy in the other animals. In the present series of experiments, 10 cc. of carbon tetrachloride was 100 per cent effective against stomach worms.

Hall and Shillinger found doses of 4, 8, 15, and 30 cc. carbon tetrachloride were 100 per cent effective against hookworms for all infested animals, four in number. There were no hookworms present in the experiment animals reported on in the present paper, so no tests could be made along this line. Doubtless carbon tetrachloride would be quite as effective when given with magnesium sulphate as when given without it. They found that doses of 12 to 48 cc. carbon tetrachloride removed 30 per cent of the nodular worms present in one series of animals; doses of 15 to 30 cc. removed 3 per cent of the nodular worms from one animal and 100 per cent from another; and doses of 4 and 8 cc. failed to remove any nodular worms. In the present series of cases a dose of 10 cc. removed all nodular worms from two infested animals. This 100 per cent efficacy with a dose of 10 cc. may be correlated on theoretical grounds with the use of the magnesium sulphate, which would tend to keep the drug from absorption and to carry it to the large intestine where the nodular worms occur. Additional experiments should be carried out to determine whether this dose will regularly maintain so high an efficacy when given with magnesium sulphate.

Hall and Shillinger found that doses of 12 to 48 cc. of carbon tetrachloride administered to one series of sheep removed 82 per cent of 801 small trichostrongyles of the genera Nematodirus, Cooperia, Ostertagia, and Trichostrongylus. This is of considerable interest, as no anthelmintic previously studied had shown any efficacy against these worms. Doses of 15 to 30 cc. removed only 3 per cent of 1,100 trichostrongyles present in two other animals; and doses of 4 and 8 cc. failed to remove any of 121 of these worms in two others, so far as could be determined by examination of the feces. In the experiments reported in the present paper, carbon tetrachloride in a dose of 10 cc. removed all of 174 small trichostrongyles present in one animal. Possibly the magnesium sulphate loosens these worms from mucus

on the walls of the digestive tract as a result of the flow of current to the lumen, thereby enabling the carbon tetrachloride to reach and destroy them. This matter deserves further study, because some of these small trichostrongyles, such as species of Nematodirus, Cooperia, and Trichostrongylus, are known to be pathogenic and are not as yet known to be amenable to treatment by any drug other than carbon tetrachloride.

Hall and Shillinger found that carbon tetrachloride in doses of 12 to 48 cc. removed from 30 to 90 per cent of the whipworms present. In the experiments reported in the present paper, 10 cc. of carbon tetrachloride failed to remove the whipworms present.

Hall and Shillinger did not find carbon tetrachloride of value in removing tapeworms from sheep, the drug removing one worm and leaving large numbers. Its much greater value in the experiments reported in the present paper may be correlated with the use of magnesium sulphate. In personal conversation the writers have been told that magnesium sulphate alone will remove some tapeworms from sheep, although no experimental evidence along this line is available.

In the experiments reported here, carbon tetrachloride in doses of 10 cc. simultaneously administered with 4 ounces of magnesium sulphate dissolved in 250 cc. of water, proved 100 per cent effective against stomach worms, nodular worms, and small trichostrongyles. This finding confirms previous work of the writers on stomach worms, and indicates an even higher efficacy than previously reported for the drug against nodular worms and small trichostrongyles. The increased efficacy is apparently correlated with the simultaneous administration of magnesium sulphate. At present it can not be said just how safe carbon tetrachloride is for ruminants.

Carbon tetrachloride was 33 per cent effective against tapeworms, an increase over the very low efficacy previously reported by the writers against these worms; and this increase also may be correlated with the use of magnesium sulphate. It failed entirely against whipworms, only one of these being present. Such a result might be expected in many cases in spite of excellent results in individual cases, because the drug does not always enter into the cecum where these worms are.

As regards the toxicity of carbon tetrachloride for sheep, Hall and Shillinger reported that a lamb weighing 15 kg. (33 pounds) was down and apparently dying on the fourth day after receiving 30 cc. of carbon tetrachloride in 2 ounces of castor oil. This is a large dose for so small an animal, although dogs would tolerate much larger doses. As the authors have noted elsewhere, ruminants are not so tolerant of many volatile anthelmintics as are carnivores.

No bad effects were noted for the 10 cc. dose reported in the present paper, but on post-mortem examination the kidneys of No. 7c were found to present a pathological appearance, and on microscopic examination by Dr. Leigh Giltner of the Pathological Division of this bureau they showed a subacute parenchymatous nephritis with general degeneration of the epithelium. This finding suggests that some of the carbon tetrachloride may have been absorbed in spite of the presence of the magnesium sulphate. This may not be a serious matter, as the studies of Meyer and Pessôa (*35; 36*), Smillie and Pessôa (*40*), Docherty and Nicholls (*8*), and Lamson and McLean (*30*), supported by many clinical observations in human and veterinary medicine, indicate that lesions due to carbon tetrachloride of the kidneys and liver usually clear up and leave these organs in practically their original condition in a comparatively short time. The exceptions to this rule apparently occur in those cases where these organs are already damaged and unable to tolerate further insult.

LARGE-SCALE TESTS OF REPEATED DOSES OF CARBON TETRACHLORIDE FOR WORMS IN SHEEP

To obtain further information on the possibility of using carbon tetrachloride as a control measure for worms in sheep, a flock of 23 sheep was put on monthly treatments. At the first dosing in October, 1923, 17 of these sheep were given 5 cc. of carbon tetrachloride in capsule, followed immediately by 2 oz. of a saturated solution of magnesium sulphate as a drench. Two sheep died because the solution of magnesium sulphate entered their lungs and toxic action apparently followed its absorption by them. The amount of solution appears to have been too small to cause death by asphyxiation; much larger amounts of water or normal saline may be introduced into the lungs of animals, as a rule, without bad effects. Magnesium sulphate is known to be toxic when administered intravenously or even in saturated

solutions by mouth in rare instances. The use of intraperitoneal solutions of magnesium sulphate to kill dogs has recently been advocated, and the writers have found it a comparatively rapid and painless method, the most striking post-mortem lesions consisting in a vascular engorgement of the blood vessels of the viscera of the abdominal cavity.

In view of the bad effects on these sheep, the remaining six were each given 6 cc. of carbon tetrachloride with two No. 10 capsules of pure magnesium sulphate. Some of these animals appeared quite uncomfortable for a few minutes after dosing and showed a rapid respiration.

In further experiments some changes were made in the number of sheep and the individuals used in this flock, and the dose adopted for routine use was 5 cc. of carbon tetrachloride in a hard capsule, No. 13, holding a little more than 5 cc., together with dry magnesium sulphate in an equivalent bulk, about 5 cc., in the same-sized capsule. This treatment has continued now for 5 months since the first treatment, a total of 6 treatments, and fecal examinations show that it is of value in controlling infestations with various worms. It is planned to continue such monthly treatment for about a year, at the end of which time post-mortem examinations, together with the tables of weights for the period of the experiment, will show something more definite as to the value of the treatment.

TEST OF ARSENIC FOR REMOVING WORMS FROM HORSES

PROTOCOL

Horse No. 233, weighing 735 lbs., was given the following preparation: Arsenic, 30 grains; calomel, 60 grains; animal charcoal, 80 grains. This was administered in a hard gelatine capsule in the morning after fasting the animal from noon of the previous day, and was followed by a quart of water administered as a drench, with no feeding until 3 hours after treatment. The manure was examined daily for the next 6 days and no worms were found. The animal was then given copper sulphate as noted in the next experiment and, as noted in that experiment, was found to have numerous worms (*Habronema* spp., *Strongylus* sp., and cylicostomes) present post-mortem. The conclusions are similar for both experiments.

DISCUSSION

Although arsenic and combinations of arsenic and copper sulphate are effective in controlling stomach worms in sheep and goats, and the combination is quite effective in controlling tapeworms, hookworms, and to some extent nodular worms in these animals, arsenic in the dose given and as administered does not appear to be promising as an anthelmintic for worms in horses, although it has long been recommended and used for this purpose. Winslow (*43*) states: "Large single doses of arsenious acid (℥ss) are sometimes given with calomel (℥i) and aloes (℥iv) in a ball to horses to kill roundworms." This is the dose of arsenic tested here. As the horse used had no ascarids, no conclusions are possible on the value of arsenic against these worms in the horse; but other tests with arsenic suggest that carbon bisulphide and carbon tetrachloride, and perhaps chenopodium, are superior to arsenic for this purpose.

TEST OF COPPER SULPHATE FOR REMOVING WORMS FROM HORSES

PROTOCOL

Horse No. 233, weighing 735 pounds, was given 8 gm. of copper sulphate in 192 cc. of distilled water, the solution being administered as a drench. This was given in the morning after fasting the animal from noon of the previous day. The horse was not fed until an hour after treatment. The manure was examined daily for the next 9 days and no worms were found. The animal was killed on the ninth day and worms collected from it as follows: Numerous specimens of *Habronema* spp.; 221 *Strongylus* sp. collected and counted and an additional 104 estimated as present, or a total of 325 *Strongylus* sp.; 122 cylicostomes collected and counted and an additional 1,088 estimated as present, or a total of 1,210 cylicostomes.

DISCUSSION

The treatment must be regarded as ineffective, although it can not be concluded that some worms of the genus Habronema might not have been destroyed by the copper sulphate, for, as pointed out above, when worms in the stomach are killed by any anthelmintic, only the worms near the pylorus may be expected to escape digestion. In the long journey through the large intestine

of the horse such small worms as Habronema may be macerated beyond recognition.

TESTS OF NOVARSENOBENZOL ON STRONGYLES IN VERMINOUS ANEURISMS OF THE HORSE

PROTOCOL

Horse No. 223, an old animal weighing 710 pounds, was given daily intravenous injections of novarsenobenzol (Billon) in amounts of 3.6 gm. dissolved in 40 cc. of sterile distilled water, slowly injected into the jugular vein. The animal showed no pronounced symptoms from the injections until the fourth day, when it became uneasy 5 minutes after the injection, staggered and almost fell, breathed hard, and passed watery feces and some gas. It tried to eat but stopped to look at its flanks and the skin quivered over the flank region. The respiration was 68 and shallow. Apparently the drug caused cramps and extreme peristalsis. The animal was killed 3 days after this last dose, and an examination was made for worms in an aneurism of the anterior mesenteric artery which was about 7 or 8 inches long and involved the nearby arterial branches. Embedded in the thrombus and in the thickened arterial walls were 14 live specimens of *Strongylus vulgaris* in a late larval stage, all ensheathed in a somewhat loose cuticle. One additional specimen could not be definitely identified since it appeared to be either a dead and partially disintegrated strongyle or a shed cuticle filled with some dark material.

In a previous paper Hall and Shillinger (*20*) have reported failure to destroy these worms with intravenous injections of tartar emetic and of carbon tetrachloride. So far no treatment is known for destroying these worms; and, on the available evidence, no promising results can be expected from tartar emetic, carbon tetrachloride, or novarsenobenzol.

GENERAL SUMMARY AND DISCUSSION

EXPERIMENTS ON DOGS

ASCARIDS.—A mixture of carbon tetrachloride, 3 parts by volume, and chenopodium, 1 part by volume, given at a dose rate of 0.3 cc. per kilogram with one-eighth to one-half grain arecoline hydrobromide according to the size of the dog, shows a rather high efficacy, 90 per cent, in removing ascarids from dogs, but this efficacy is slightly less than that usually obtained with chenopodium or carbon tetrachloride given without arecoline hydrobromide. The number of infested animals and of worms present in the experiment was not sufficient to warrant the conclusion that the slight loss in indicated efficacy would be sustained in a larger series of cases.

The simultaneous administration of magnesium sulphate with a therapeutic dose of chenopodium at the rate of 0.1 cc. per kilogram gives an indicated efficacy of 94 per cent, showing that the salts do not materially diminish the efficacy of the chenopodium.

Novarsenobenzol in repeated doses by mouth was entirely ineffective against ascarids.

HOOKWORMS.—The mixture of carbon tetrachloride, chenopodium and arecoline hydrobromide removed all the hookworms from only 78 per cent of the dogs in these experiments, removing 46 per cent of the total number of hookworms present. This is a decided loss of efficacy compared to the 100 per cent efficacy of carbon tetrachloride alone or of the mixture of carbon tetrachloride and chenopodium, contraindicating the simultaneous use of arecoline hydrobromide with these drugs for this purpose.

The therapeutic dose of chenopodium for ascarids when given with salts removed the one hookworm present; no positive conclusion can safely be drawn from this test.

Benzyl-phenol in single doses of 20 and 30 grains and in doses of 20 grains one day and 30 grains the following day removed only 7 per cent of the hookworms from the experiment animals. While chemicals having a free phenolic-hydroxyl group have a certain efficacy against hookworms, as shown here, the efficacy of such compounds, including thymol, is low against hookworms in dogs.

Ethylene dichloride at a dose rate of 0.3 to 0.5 cc. per kilogram of weight of animal removed 0 to 9 per cent of the hookworms in single doses and in two doses at the rate of 0.5 cc. per kilogram removed 67 per cent from infested dogs. This efficacy is lower than that obtained by Hall and Foster (*13*) and by Hall (*15*) for chloroform, their average for single doses being 52 per cent; and is distinctly lower than the 100 per cent efficacy of carbon tetrachloride in single doses at the rate of 0.3 per kilogram. This loss of efficacy appears to be associated with the diminished

halogen content of ethylene dichloride (Cl_2) as compared with chloroform (Cl_3) and especially with carbon tetrachloride (Cl_4).

Chenopodium intravenously in a dose of 0.5 cc. failed to remove hookworms from an infested dog. In three human cases Lambert also failed to remove any hookworms from infested patients by intravenous injections of chenopodium. By intramuscular injections of chenopodium in two cases he recovered from 1.5 to 4 per cent of the indicated total of hookworms present as ascertained by subsequent treatment by mouth; a third patient passed three hookworms and was found by fecal examination still infested, but was not subsequently treated by mouth.

Ferrous sulphate in single doses by mouth removed no hookworms from dogs.

Novarsenobenzol in repeated doses by mouth removed no hookworms from one infested dog.

WHIPWORMS.—As has been repeatedly pointed out by Hall, whipworms are actually very susceptible to even quite feeble anthelmintics, in spite of the fact that it is extremely difficult to remove them with any certainty. This might be expected on theoretical grounds. The difficulty in their removal is due to the above-mentioned uncertainty of entry of anthelmintics into the cecum.

This very uncertainty, due to their location, may account for their actual susceptibility to even feeble anthelmintics. Worms in the stomach and small intestines are constantly exposed to the effects of all sorts of substances taken in with food and water or administered as drugs, and must be fairly tolerant of many substances to maintain themselves in the face of constant exposure to them. Since many of these substances are absorbed in the stomach and small intestine, and since whipworms have a protected situation in the cecum, into which only a part of the contents passing the ileocecal or ileo-colic valve ever enters, the whipworms are much less exposed to substances entering the mouth, and therefore have much less occasion to develop a tolerance for them or immunity to them. This paper reports the passage of whipworms after the administration by mouth of the combination of carbon tetrachloride, chenopodium, and arecoline hydrobromide; also of benzyl phenol and of ethylene dichloride, with no dependable efficacy shown for any of these substances.

Whipworms have been made the object of a more varied attack than have any other worms in the digestive tract, because they are so difficult to remove. The topic, recently summarized by Lambert (*29*), may be summarized further in connection with the experiments given here.

The methods of attack are as follows:

SURGICAL.—Both in human and veterinary medicine the difficulties experienced in removing these worms by anthelmintics have led to the recommendation by the physicians Berard and Vignard (*3*) and by the veterinarian Miller (*37*) that resection of the appendix in man and of the cecum in dogs be resorted to for the removal of these worms.

ORAL MEDICATION WITH REPEATED SMALL DOSES.—Hall (*11*, *14*, *17*) has recommended the use of repeated small doses of some nonirritant anthelmintic as a method of removing whipworms, and finds santonin the most satisfactory drug for this purpose in the case of dogs. He notes that Wade in human medicine has recommended the use of 2-grain doses of thymol 3 times a day over a period of 2 weeks. To date santonin has afforded fairly satisfactory results. In the experiments reported in this paper novarsenobenzol in repeated doses by mouth removed all the whipworms from an infested dog. Evidently this drug is but feebly anthelmintic, and whipworms are very susceptible to even quite weak anthelmintics, since the treatments removed all the whipworms but failed entirely against ascarids, hookworms, and tapeworms. This method of attack with repeated small doses of a nonirritant anthelmintic of relatively low toxicity is still a most promising line of attack for the removal of whipworms.

ORAL MEDICATION WITH MASSIVE DOSES.—In addition to the use of repeated small doses, one solution of the problem of getting an anthelmintic into the cecum in contact with whipworms, is the use of single massive doses of a relatively nontoxic substance. In human medicine the latex of a fig, *Ficus laurifolia*, native in South and Central America, appears to be such a drug. In the present experiments the use of iron sulphate has been tested on the assumption that it might be effective when administered in large doses. The results indicate that it is not valuable in doses of from 28.2 to 87.8 grains, since it failed to remove whipworms from five dogs and removed only 4 per cent of these worms from one dog. The fig latex referred to above has the disadvantage of not being available outside of the areas in

which the fig grows, as the latex does not stand shipment, and it also has the disadvantage of being toxic in some cases. This method of attack promises considerable usefulness if a drug of low toxicity, capable of administration in bulky doses, that will stand shipment and will keep well, can be developed.

INTRAMUSCULAR AND SUBCUTANEOUS INJECTION.—This method of attack for whipworms appears to have been tested first by Strong (*42*), who found thymol and chenopodium intramuscularly of no value with human patients. More recently Lambert (*29*) has tested chenopodium intramuscularly on human patients and recovered 22 whipworms in one case. In the present experiments this method of treatment with chenopodium gave entirely negative results on two dogs. Apparently it will remove whipworms in some cases, but the action of the drugs tested does not seem dependable. Novarsenobenzol tested subcutaneously in one case gave negative results. This appears to be the first test of subcutaneous medication for this worm. Possibly better drugs will be found and will give a dependable action warranting their use in intramuscular injections. Whipworms appear to feed on blood or serum and it is theoretically possible to poison them by drugs introduced into the system by intramuscular or intravenous injections. The experimental and clinical evidence to date sustains this possibility, but does not sustain the idea that the action is dependable for the drugs tested.

INTRAVENOUS INJECTIONS.—Intravenous injections for removing whipworms appear to have been tested for the first time by Lambert (*29*) on three human patients, all of them passing whipworms, from 11 to 30 in number. This treatment, so far as tested, cannot, however, be expected to offer dependable action on dogs. Intravenous injections removed one whipworm from each of two infested dogs, showing that the intravenous injection of an anthelmintic will remove whipworms; but after treatment 6 worms remained in one case, and 14 in the other. If the treatment were safer it would still promise much of value, but the production of syncope in the human and other cases, and of death in one dog, indicate rather definitely that chenopodium intravenously is too dangerous for use. There remains, however, the possibility of finding a safe drug which will be effective against whipworms when administered intravenously, and this deserves investigation. This possibility has been given some consideration in the experiments with novarsenobenzol and tartar emetic reported here, but negative results with five dogs indicate that these drugs do not fulfill the necessary conditions as regards efficacy.

RECTAL INJECTIONS.—For the removal of whipworms from sheep, Brumpt, as reported by Railliet (*38*), has used rectal injections of 1 to 1.5 liters of water containing a thymol emulsion, at the rate of 1 gm. thymol to each 3 to 5 kg. of weight of animal. The efficacy of this method is not certain. Hall and Shillinger (*22*) have tested the use of rectal injections of various drugs for the removal of heterakids from the ceca of chickens, and found that chenopodium in doses of 0.1 cc. in 5 cc. of a bland oil removed 90 per cent of the worms. Subsequently, Freeborn (*9*) has reported that nicotine sulphate solution, containing 40 per cent nicotine in a dilution of 1 cc. to 200 cc. of distilled water, administered in doses of 10 cc. by rectal injection, removed 85 per cent of the heterakids. The ceca of birds are much more accessible by rectal injections than is the cecum of mammals.

The foregoing shows that whipworms have been attacked by surgical measures, by single doses of the ordinary anthelmintics by mouth, by repeated small doses of non-irritant anthelmintics of relatively low toxicity, by single massive doses of anthelmintics of relatively low toxicity, by intramuscular injections, by subcutaneous injections, by intravenous injections, and by rectal injections.

TAPEWORMS.—It has been shown by Hall and Shillinger (*23*) that arecoline hydrobromide, given as recommended by Lentz (*32*), in critical tests removes all of the tapeworms from dogs in the majority of cases, and fails to remove any in a minority of cases, so far as tested on seven dogs. It is shown in this paper that when given simultaneously with therapeutic doses of a mixture of carbon tetrachloride and chenopodium, arecoline hydrobromide apparently suffers a serious loss of efficacy, failing to remove any tapeworms from two infested animals. In view of the uncertainty of action of tapeworm remedies in general, not too much may be judged from the failure here, but the combination in question fails to warrant the expectations based on the action of its individual constituents.

An extensively advertised proprietary remedy for removing tapeworms from dogs removed only 9 per cent

of these worms from one infested dog. The only conclusion warranted in this case is that this preparation will not always remove all of the tapeworms from infested dogs, but this conclusion applies to tapeworm remedies in general.

Of the other substances tested here for their effect on various worms, chenopodium and magnesium sulphate simultaneously, benzyl-phenol, ethylene dichloride, ferrous sulphate and novarsenobenzol by mouth failed to remove any tapeworms, as did chenopodium and novarsenobenzol intravenously.

INFLUENCE OF MAGNESIUM SULPHATE ON TOXICITY OF ANTHELMINTICS.—An experiment on four dogs showed that three animals survived a lethal dose of chenopodium when given with an adequate dose of magnesium sulphate, and one animal died. The survival of three animals confirms in the case of chenopodium the possible protective action of magnesium sulphate noted by Hall and Shillinger (*25*) in the case of carbon tetrachloride. At the same time it is noted that the protective action is not entirely a function of the salt action of magnesium sulphate in inhibiting absorption by virtue of the creation of a flow of fluids from the wall of the digestive tract to the lumen, because Hall has shown that purgatives like castor oil and calomel exert a protective action against lethal doses of such drugs as chenopodium and male fern. The protective action is therefore partly due to purgation alone.

EXPERIMENTS ON MONKEYS

Carbon tetrachloride given with magnesium sulphate, will remove hookworms and nodular worms from monkeys, but the exact efficacy is not known.

EXPERIMENTS ON SHEEP

The present findings confirm the previous findings of Hall and Shillinger (*25*) on the value of carbon tetrachloride in removing various worms from sheep, and indicate that the simultaneous administration of magnesium sulphate with this drug may increase its efficacy. Doses of 10 cc. of carbon tetrachloride with magnesium sulphate are apparently 100 per cent effective in removing stomach worms (*Haemonchus contortus*), nodular worms, and small trichostrongyles, and on the basis of the writers' previous work on carbon tetrachloride for hookworms in sheep, and carbon tetrachloride and magnesium sulphate for hookworms in dogs, will probably be 100 per cent effective in removing hookworms from sheep. The same dose shows an efficacy of 33 per cent in removing tapeworms from sheep, and in repeated doses would presumably serve to clear out these worms. The dose used failed to remove whipworms from sheep, as might be expected to occur in most cases for reasons given above in the discussion of whipworms in dogs.

ARSENIC.—A test of arsenic in a single dose of 30 grains indicates that as given it is of no value in removing stomach worms (*Habronema* sp.), palisade worms (*Strongylus* sp.) and cylicostomes from the horse.

COPPER SULPHATE.—Copper sulphate (8 gm.) in solution was entirely ineffective, apparently, in removing stomach worms, palisade worms, and cylicostomes from the horse.

NOVARSENOBENZOL INTRAVENOUSLY.—Daily intravenous injections of novarsenobenzol for 4 days, totaling 14.4 gm., failed to kill strongyles in the anterior mesenteric artery. Tartar emetic and carbon tetrachloride intravenously have also been previously reported by the writers as failing to kill these worms in aneurisms.

GENERAL CONCLUSIONS

A mixture of carbon tetrachloride 3 parts by volume, and chenopodium, 1 part, at the rate of 0.3 cc. per kilogram accompanied by ⅛ to ½ grain arecoline hydrobromide, a combination which should on theoretical grounds be valuable against ascarids, hookworms and tapeworms in dogs, was found to maintain the rather high efficacy of 90 per cent against ascarids, but was considerably less efficacious against hookworms as compared with its constituents without the arecoline hydrobromide, removing only 46 per cent; and it failed entirely to remove tapeworms. The arecoline hydrobromide appears to diminish the efficacy of the other drugs against hookworms, possibly by its very rapid purgative action, purgation commonly occurring in from half an hour to an hour, and, so far as can be judged from two cases, the efficacy of the arecoline hydrobromide against tapeworms suffers a dimunition from the presence of the two other drugs.

Benzyl-phenol proved only slightly anthelmintic for hookworms, removing only about 5 per cent of the hookworms present.

Ethylene dichloride proved only slightly effective against hookworms when given at a dose rate equivalent to the therapeutic dose rate for carbon tetrachloride.

Ferrous sulphate in large doses showed very little efficacy against whipworms and is evidently not dependably effective in single doses for removing these worms.

Chenopodium intramuscularly failed to remove whipworms from two infested dogs. Intravenously it removed only one whipworm from each of two infested dogs, showing that the drug has some anthelmintic action when thus given, as Lambert had found, but also showing a lack of dependable action even in dangerous doses.

Novarsenobenzol subcutaneously failed to remove any whipworms from the one dog on which the drug was thus tested. Intravenously in single dose to one dog and in two doses to another it failed to remove any whipworms.

Tartar emetic in single dose intravenously to three dogs failed to remove any whipworms.

A proprietary remedy for tapeworm in dogs removed only 9 per cent of the tapeworms present.

Magnesium sulphate simultaneously administered with lethal doses of chenopodium to dogs protected them from the toxic effects of the chenopodium, three out of four animals surviving the dose. Hall has shown that castor oil or calomel will protect dogs against the toxic effects of lethal doses of such drugs as chenopodium and male fern, from which it appears that the protective action of magnesium sulphate is due not only to its salt action but also to its purely purgative action. Our experiments also show that magnesium sulphate simultaneously administered with therapeutic doses of chenopodium does not diminish the efficacy of the chenopodium against ascarids, and suggest that the same is true as regards effect on hookworms. The writers have previously shown that magnesium sulphate simultaneously administered with carbon tetrachloride does not diminish the efficacy of this drug against hookworms.

Carbon tetrachloride, given with magnesium sulphate, will remove hookworms and nodular worms from monkeys, but the exact efficacy is unknown.

Carbon tetrachloride given to sheep in doses of 10 cc. followed immediately by 128 gm. of magnesium sulphate shows 100 per cent efficacy against stomach worms, nodular worms, and small trichostrongyles. Previous evidence from other experiments indicates that it is also 100 per cent effective against hookworms. When thus administered with magnesium sulphate there is a marked increase in efficacy against tapeworms, this treatment removing 33 per cent of these worms instead of being almost entirely ineffective. A flock of sheep is now being treated with monthly doses of 5 cc. of carbon tetrachloride and an equivalent bulk of dry magnesium sulphate to determine the value of such repeated treatments in controlling worms in sheep.

Arsenic in the 30-grain dose ordinarily recommended for worms in horses apparently failed entirely to remove any stomach worms, palisade worms, or cylicostomes from a horse. Copper sulphate (8 gm.) in solution apparently failed entirely to remove any stomach worms, palisade worms, or cylicostomes from a horse.

Novarsenobenzol intravenously for 4 days for a total amount of 14.4 gm. failed to kill strongyles (*Strongylus vulgaris*) in a verminous aneurism in a horse.

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STUDIES ON THE INHERITANCE OF EARLINESS IN WHEAT [1]

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INTRODUCTION

The studies reported in this paper were undertaken primarily to determine the inheritance of earliness in wheat in connection with breeding for earlier varieties. As the cross under observation was intended purely for economic purposes, only general notes were taken on the F_1 progeny. But in regard to the F_2 and F_3 progenies, earliness notes were taken on individual plants and families.

The investigation of the problem of early maturity in plants has engaged for many years the active interest of geneticists and plant breeders. The value of such investigation has been emphasized by the practical importance of developing varieties of crop plants suitable for the early market or of such precocity as to escape unfavorable conditions induced by climate or crop pests.

In general, the behavior of the hybrid material in the different studies reported has been similar. In the F_1 progeny, time of heading or maturity has been found to be intermediate between that of the parents, with the means or the hybrid inclining toward one or the other parent. In the F_2 the tendency has been for the intermediate condition to be maintained but with a range of variation almost covering the extremes of both parents, and both with and without the grouping into classes showing definite segregation. In subsequent generations the segregation into distinct classes becomes more and more marked as homozygosity is approached.

REVIEW OF LITERATURE ON INHERITANCE OF EARLINESS

EXPERIMENTS WITH WHEAT

William Farrer (*6*)[3] early observed that the progeny in the F_1 generation was intermediate between the parents, and that there was no difference in this respect in the reciprocal of a cross. In the F_2 he found rather wide variation in time of ripening.

Biffen (*2*) found that the F_1 produced ripe grains well along toward the date of ripening of the late parent of an interspecific cross between an early (Polish) and a late (Rivet) wheat. In the F_2, although all plants flowered simultaneously, many plants ripened at the same time as the early parent, while some were almost ripe and still others green. He concluded that earliness is dominant.

Fruwirth (*8, p. 176, 238, 314*) believes the early ripening to be partially dominant in wheat, rye, and barley.

Thompson (*15*) made numerous crosses between eight varieties of wheat, ranging from very early to late maturing. In ten F_1 crosses with the late parent the ripening period of most plants was near the mean of the late parent. In the F_2 most individuals were intermediate, with no indication of "heaping up" except in the intermediate position, but the range of variation extends nearly from that of the earliest to that of the latest of the parent varieties. He found, as did Biffen (*2*), that earliness and lateness are inherited independently of other characters. He also made the interesting observation that the earliest plants of an F_2 population frequently were quite as large and productive as the latest grandparent.

Freeman (*7*) made crosses between durum and common white wheats, as well as between two common wheats. He found that the average date of first heading in both F_2 and F_3 progenies in every case was intermediate but nearer to that of the late parent. In the durum cross, there was an extension of range much beyond that of the late parent.

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[3] Reference is made by number (italic) to "Literature cited," p. 347.

In a cross between Sonora and Turkey wheats, Bryan and Pressley (*3*) found the F_1 progeny almost exactly intermediate between the parents in time of first heading. In the F_2 generation the range of variation was but slightly greater than that of both parents but inclined toward the late parent. In the F_3 progeny several early plants were isolated which were almost as early as the early parent. In the F_4 progeny, the range of heading dates of a somewhat larger number of early plants was considerably narrower than that of the early parent.

EXPERIMENTS WITH OTHER CROPS

In studies of earliness in peas, Mendel (*1*, *p. 337*), found that the time of flowering of the F_1 generation was intermediate between those of the parents. Keeble and Pellew (*11*), in their work with time of flowering and stature of peas, concluded that lateness was dominant over earliness and that apparently there is a relation between the morphological and vegetative characters and the period of flowering in plants.

Leake (*12*), in studying the blooming time of cotton, found the F_1 generation intermediate, while the F_2 progeny formed a regular curve with the mean nearest the late parent.

Emerson and East (*5*) found that the time of first exposure of the anthers was distinctly intermediate in the F_1 generation of a cross between a dent corn (late) × pop corn (early). In the F_2 progeny some of the plants were nearly as early as the earliest of the early parent and others as late as the late parent. Ten families held the same relative order of ripening as of flowering, while the parent varieties were further apart in ripening than in blossoming.

Hoshino (*10*), working with rice, found that emergence of the head in the F_1 generation inclined to that of the early parent. In the F_2 progeny he found two segregation groups, with the minimum frequency midway between the variation ranges of the parents, and with the early group the larger. Further segregation groups appeared in the F_3 and F_4 generations. He explains his results by a 3-factor hypothesis.

Caporn (*4*), in reporting on the inheritance of earliness in an oat cross, found the F_1 progeny intermediate between the parents in ripening. In the F_2 progeny much variation was observed, but in the F_3 generation the variation did not extend so far as that of the parents.

INDICES OF EARLINESS IN CEREAL CROPS

The indices used in the studies on the inheritance of earliness in the cereal crops have varied considerably. Farrer (*6*) took as his index the change in color of the peduncle immediately below the spike. Biffen (*2*) used the fully ripe condition, as indicated by yellow straw and glumes and hard grain, although in order to get a comparable reading of his populations he also used half ripe, as indicated by yellow awns, other parts yellowish green, and the grain soft. His third class was green throughout. Thompson (*15*) used the term "fully ripe" for the time when the central kernels of the spike reach the dough stage. Caporn (*4*) in studies on oats considered fully ripe to indicate disappearance of the last trace of greenness from the tips of the paleae.

Freeman (*7*) used as the index of earliness in wheat the date of appearance of the first head on each plant, as did also Bryan and Pressley (*3*). Hoshino (*10*), in rice, used the shooting time, i. e., the appearance of the first spikelet above the sheath of the inflorescence. Emerson and East (*5*) used the time of first exposure of the anthers in corn.

In comparing earliness of varieties of cereals in agronomic experiments, the progression of the variety toward maturity usually is designated by several different stages. In wheat these generally include first heading, fully headed, first ripe, and fully ripe. There is usually quite close correlation between these different stages in the development of the plants. Then, in all but the exceptional cases, the stage that will be most sensitive, that is, that will show the greatest divergence in time of appearance, should most accurately show the relative earliness of varieties or strains. It is well known, and also has been the writer's experience in agronomic note-taking for a number of years, that the relative difference in earliness at first heading is considerably greater than at maturity. The rate of development of the wheat plant is more rapid at the time of ripening than at the time of heading. Ripening often is more or less abnormal also, owing to the effect of high temperatures, drought, etc.

Harlan (*9*, *p. 8*, *9*), found in his studies on barley that the evidence seemed all in favor of the first emergence of the awn as a character possessing heritability equal to that of most plant characters. He also states that the date of ripening is subject to influences induced by climatic factors. In awned varieties of wheat

or barley the first appearance of the awn is a more definite stage than the first appearance of the spike. In awnless varieties emergence of the tip of the spike is practically as definite as the first appearance of the awn in an awned variety.

AGRONOMIC NOTES ON EARLINESS IN WHEAT

In the studies herein reported, curves have been prepared from the data on heading and ripening of varieties of wheat in the wheat classification nursery at Davis, Calif., in the year 1922, and also from Chico, Calif., in 1920, for comparison. Both seasons were normal, except at Chico where the temperature was relatively high during the period of ripening. Figure 1 shows the curves of first heading, all headed, first ripe, and all ripe for 29 representative varieties for the Davis nursery in 1922.

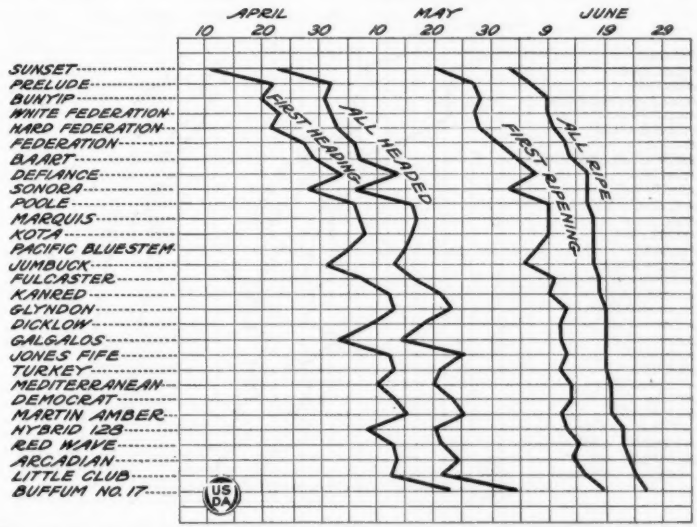

FIG. 1.—Curves showing dates of first heading, all headed, first ripening, and all ripe, for 29 representative varieties of wheat grown in the wheat classification nursery at the University Farm, Davis, Calif., in 1922

All varieties in the classification nursery are pure lines, and the date of first heading has been fixed as the time when the tips of the first 3 or 4 heads appear in the pure-line row. The date recorded for the stage of development termed "all-headed" is the time when all heads not influenced by border position were fully exerted. The plants favored by position were those included within the last six inches at each end of the row. Frequently the plants in this location would develop nearly as rapidly as those in the remainder of the row, while at other times their growth may be definitely retarded.

The curves show a comparatively wider divergence of the dates of first heading and all headed than of the dates when the first heads ripened and when all were ripe. The difference, for example, in the date of first heading of Marquis and of Sunset was 25 days; in date of first ripening it was 17 days; and in date when all were headed it was 15 days. Figure 1 shows that most of the varieties present a relatively similar divergence. Little Club, when compared with Arcadian, is an exception in that it heads earlier but ripens later than Arcadian. Farrer (*6*) also made the observation that the variety of wheat that heads first is by no means the first to ripen. It may be noted that this behavior is exceptional. Club varieties are abnormal in their behavior since they require a longer fruiting period than the common wheats.

Figure 2 gives heading and ripening data for the same 29 varieties grown at Chico, Calif., in 1920. In this season the difference in time of first heading of Sunset and Marquis was 28 days; in

date of first ripening, 16 days; and in date when all were ripe, 13 days. In general, the Chico data agree very closely with the Davis data, except that the high temperatures reduced the length of the ripening period.

EXPERIMENTAL PROCEDURE AND DATA

The index of earliness used in the studies reported in this paper was the date of emergence of the tip of the first spike. This is believed to be the most dependable index for studying earliness in cereals under California conditions.

PARENT VARIETIES

The cross used in this study was between a pure line of Marquis and a pure line of Sunset, the latter being the earliest of the Australian varieties of wheat introduced thus far. In a collection of over 1,400 foreign and domestic wheats, including a considerable number of early wheats from India, grown in 1920 at the United States Plant Introduction Station, Chico, Calif., Sunset was the first variety to head. Marquis is considered a moderately early variety throughout the northern spring-wheat belt of the United States and Canada, but when grown in California it is quite late in comparison with Sunset.

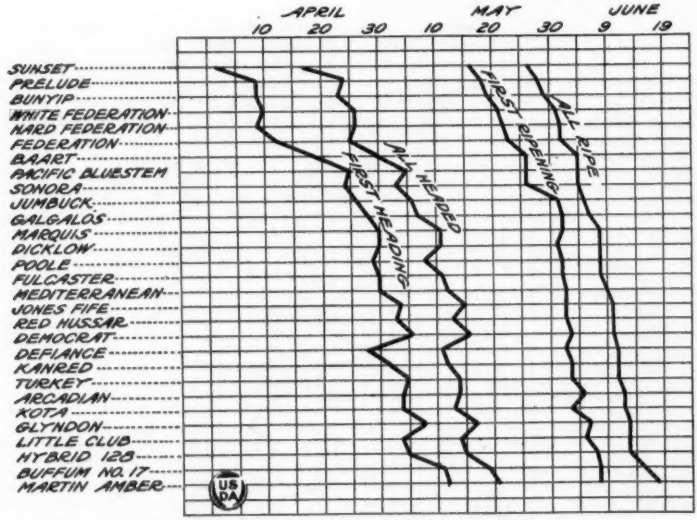

FIG. 2.—Curves showing dates of first heading, all headed, first ripening, and all ripe, for 29 representative varieties of wheat grown in the wheat classification nursery at the Plant Introduction Station, Chico, Calif., in 1920

The date of first ripening was taken as the time when the tip spikelets of the 3 or 4 earliest heads in the row began to take on the natural color of maturity and their kernels began to show a definite stiffening or hardening. It may be added that the color of maturity of the chaff varies. In white-glumed varieties it is nearly white to white or yellowish. In brown-glumed varieties it is yellowish brown to brown. The date on which the plants were recorded as all ripe normally is the time when all heads in the row were fully ripe, except those subject to border effect, which were not considered. At this stage the kernels were hard and the color of the glumes had changed to normal at maturity.

P_1 MATERIAL AND F_1 PROGENY

The cross, Marquis × Sunset, and its reciprocal, was made in 1920 at Chico, Calif. In making the cross two spikes from each variety were used, and the pollen was obtained at random from each of the parent rows. Nine apparently crossed kernels were obtained from the cross Marquis × Sunset and 21 kernels from the reciprocal Sunset × Marquis.

The F_1 generation was grown at Chico. The date of first ripening of this population as a group was noted to be slightly earlier than that of the Marquis parent.

TABLE I.—*Frequency table showing distribution of the parents and the F_2 progeny of the Marquis×Sunset and Sunset×Marquis crosses by dates of first heading in head rows at the University Farm, Davis, Calif., in 1922*

TABLE I.—*Frequency table showing distribution of the parents and the F_2 progeny of the Marquis×Sunset and Sunset×Marquis crosses by dates of first heading in head rows at the University Farm, Davis, Calif., in 1922*

Marquis×Sunset and Reciprocal	Dates of first heading																																	Totals
	April																							May											
	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	1	2	3	4	5	6	7	8	9	10	
Sunset Parent						1		4	2	1	2	1	7	4	1	4	1	1	1											3	8	6	5	6	
Marquis Parent																																			
Marquis×Sunset:																																			
20166A1														4	6	2	5	1	3	2	1		1			4		1							30
A2												1		3	2	4	1	3	1	4	3	2	4		1		2	3	1	3		1				39
A3	1			1								1	1		4	3	3	2		3	4		1			1	3	2							30
A4												3	5	1	10	4	3	5				1	2	1		1	3	1	1	1					42
B1												1	3	7	5	2	1		1	2	2	1	1	2	4			2		1					35
Sunset×Marquis:																																			
20167A1											2	3	3	1	5	4	1	1	1				1	3	1	1	2	5			1				35
A2												3	2	2	4	5	6	4		2	1		1		1	2		3							36
A3										1	2	2	2	8	4	6	1	1		2			1			2		1							33
A4								1	1	1		2		3	5	2	3	2		1	1		1		2		1	2	1						29
A5													3	4	3	2	4	2	2	2	2			2	1	2		1							30
A6									1		2	2	1	3	4	4	1	4	1	1				2	1	2	2		1						32
A7											1		2	4	3	5	2	1	3	1					2					1					25
A8									1			3	5	1	6	5	3	2					1	1	1	1			1						31
A9								1		1			4	4	6	5	4	2	2	2			1	1	1	3	3								41
A10									1	1	1	3		2	3	4	6	3	2	1	2			1					1		1				32
A11												2	3	5	8	4	5	2	1		2	1		3	3	3	2	1							45
A12								1	1		2	1	7	4	4	5	4	2							3	3	1	1	2		1				39
A13											1		3	3	5	7	6	3	4	2	1	1	1		2	3									42
A14							1		1		1	3		1	3	5	3	2		1			1	3			1	1							27
B1										1		1	4	8	2	3	5	2	4	4	2				1		2	2							41
B2									1		2		4	2	4	2	2	3	2		1		1		1	1			2						28
B4										1	2		2	2	1	3	3	1	2		5		1	6	1	1	1		1	1	1				35
B5												4	2		5	2	5	4		1		2	1	1	2	4		2							35
Totals	1			1			1	3	7	6	16	35	56	72	102	88	77	52	29	31	28	8	19	27	25	34	23	28	11	7	3	2			792

F_2 PROGENY

In the fall of 1922 the kernels from one average head of each of the F_1 plants were sown at the University Farm, Davis, Calif. The kernels were spaced 2 inches apart in the row. The date of emergence of the first head on each plant was noted. Seven hundred and ninety two hybrid plants, representing 23 families, were tagged for first heading. A frequency table of these data, showing the variation in time of heading, is given in Table I, and a frequency polygon is shown in Figure 3. The frequency curve shows a so-called "heaping up" of individuals into a large group of fairly early plants and a smaller group of rather late plants, with a distribution covering almost the entire range of the parents. A few plants were earlier than the earliest plants of the early parent, while only one was as late as the latest plants of the late parent. Considering the family progeny in each row, in practically every case there was a definite segregation into an early group and a late group, with a distribution of each closely approximating a normal frequency curve. A combination of all families represented shows that there is some overlapping of the two distributions. If an arbitrary division between early and late groups is made at April 28, which is the minimum frequency class in this part of the curve, and half of the 8 individuals therein are assigned to each group, there will be 609 plants in the early group and 183 plants in the late group, which is a ratio of 3.07 : 0.93. This indicates a one-factor difference for earliness in the two varieties. The deviation from the 3:1 ratio probably is not significant, for it is less than twice the probable error, ± 0.04.

Figure 3 gives the curve of the maximum daily temperature during the heading period of the cross. The maximum temperature gives a fairly good measure of the prevailing daily temperatures at this time. These data are included to show that low temperatures were not the cause of the low frequency of first heading on April 28, and the consequent bimodal form of the curve.

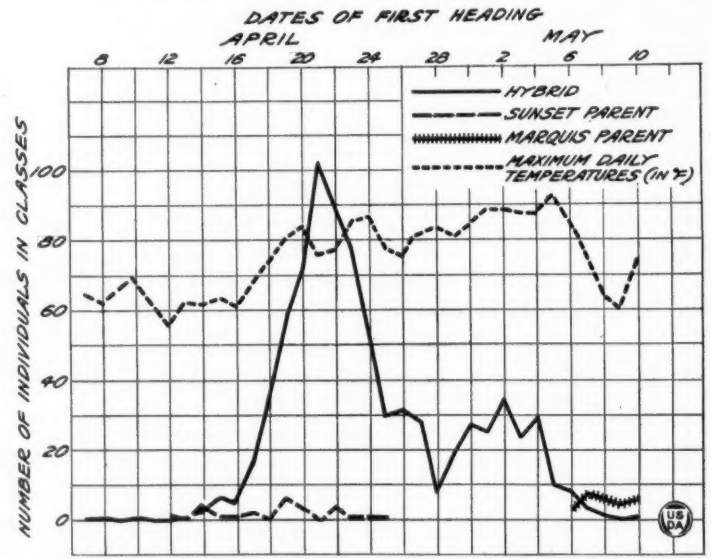

FIG. 3.—Curves showing the frequency distribution of the F_2 hybrid population, the Sunset parent, and the Marquis parent, by dates of heading, and the maximum daily temperature for the heading period in degrees Fahrenheit, at the University Farm, Davis, Calif., in 1923

F_3 PROGENY

The sowings for the F_3 progeny were made in the same way as those for the F_2. One head from each of the F_2 plants was harvested and the kernels were sown 2 inches apart in rows 5 feet long and 16 inches apart. The index for earliness, however, was obtained in a different manner. The date of first heading for each individual plant in each row was not noted, but the appearance of the first head

in each row was recorded, as well as the date when the last head made its appearance. This gave the date of first heading of progeny from each F_2 plant, as well as the interval of time required from first heading until all plants were fully headed.

As a wheat plant is considered a unit in heredity, any spike from that plant will exhibit in its homozygous progeny the characters of its parent. A homozygous head row from a recessive late plant will begin heading late; a homozygous early head row will begin heading early; and a heterozygous head row will begin heading early, as it contains both early and late plants. In the F_3 generation, therefore, a similar distribution for first heading should be obtained as in the F_2. Table II gives the frequency distribution of date of first heading of the F_3 head rows.[4] There is a sharp segregation into an early group and a late group almost identical with that in the F_2 generation.

If the division is made between the April 2 and April 3 dates of first heading in the F_3, the totals in the early and late classes are 607 and 184, respectively, or practically the same as were placed in the early and late classes in the F_2. These totals are not made up entirely of the same individuals as made up the early and late classes in the F_2, however, as about 4 per cent of the segregates have shifted back or forth with respect to date of first heading.

USE OF HEADING DATA AS A METHOD OF ANALYSIS OF F_3 ROWS

A further analysis of the F_3 population was made to determine, if possible, the segregation for rows homozygous and those heterozygous for duration of heading.

In taking agronomic notes on pure-line varieties it has been observed that within a group, such as common wheat, club wheat, etc., the period from first heading to fully headed is fairly uniform and constant (figs. 1 and 2). On account of a number of factors, such as lower temperatures, shorter period of daylight, etc., varieties heading early in the season require a somewhat longer period to progress from first heading to fully headed than do those coming later, but those heading at about the same time require approximately an equal time for this period of development. It should be possible in segregating hybrid material, therefore, to separate the early and late homozygotes from the heterozygotes by the length of time required to come from first heading to the fully headed stage. The homozygotes would require a comparatively short time to complete the heading process, while the heterozygotes would require a longer time, in some cases extending over the entire season, depending upon the degree of heterozygosity.

The season of 1923 at Davis was marked by high temperatures in February and March, so that the progeny rows from the early F_2 segregates began heading in late March. A heavy rain followed by cooler weather occurred from March 30 to April 2. This retarded the heading of the late segregates and slowed down the progress of those already heading. However, most of the progeny rows from the early segregates began heading before the storm occurred, so that earliness of heading probably was not affected much in this group, and the late segregates probably were affected about equally.

USE OF TIME-TEMPERATURE EFFICIENCY UNIT AS A METHOD OF ANALYSIS

To get the most accurate measure of the time required for heading in the various rows, it was decided that temperature also must be considered, as it materially affects the rate of plant growth. High temperatures with rapid development occurred both early and late during the heading period, while in the intermediate period the rate of heading was slower. The time required for heading therefore was converted into time-temperature efficiency units. A time-temperature efficiency unit was taken arbitrarily to mean 1° F. for the duration of one hour above a certain basic temperature, below which the wheat plant probably makes no appreciable growth.

The minimum temperature of growth for the seedling wheat plant was found by Sachs (*14, p. 365*) to be 41° F., the optimum temperature, 83.7° F., and the maximum temperature, 108°. Other investigators have found that the growth of many plants ceases with falling temperatures at 40° to 43°. In growth-temperature studies with plants, 40° has been the basic temperature commonly used. It will be considered in this study also as the temperature below which little or no growth occurs in wheat. The optimum temperature was taken as 83.7°, as determined by Sachs, although it is probable that plants approaching maturity are not so sensitive to higher temperatures as seedling plants.

[4] There were 792 head rows in the F_2 (Table I), but the seed from one failed to germinate, hence 791 in F_3.

TABLE II.—*Frequency distribution of F_3 progeny in head rows by dates of first heading of early and late segregates from the F_2. (Early segregates above heavy line; late segregates below)*

Date of first heading in F_2 (1922)	Dates of first heading in F_3																																	Totals
	March							April																											
	25	26	27	28	29	30	31	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	
Apr. 7																										1										1
8																																				
9																																				1
10						1																														
11																																				1
12			1																																	3
13		1	2																																	7
14		2	3	2																																6
15		3	2	2			1																						1							16
16		1	4	7	2	1																														35
17		2	7	17	9		2		1																											56
18		1	9	30	6	7																								1						72
19		3	7	32	13	10	5	1																												102
20		2	11	46	12	22	8	1		2																1		1								88
21			7	42	16	12	7						1														1									77
22			7	24	14	15	11	2	2	2			1												1											52
23		1	3	15	9	11	8	2	2	4																										29
24				6	2	9	4																													30
25		1		8	7	4	7	1		1		1						1				1						1							28
26				6	2	12	3		1		1							1		1															8
27				1	2		1						1																							19
28				3		1	1	1	1		1		2			1		1	1	2	7	4	5													27
29							1	1		1				1					1	5	4	3	11	1	1	1	1									25
30								1										1		1	8	2	8	2	2	2										34
May 1																				2	1	3	7	1	6	6	3									23
2																		2		1	1	2	5	2	2	3	1		1	1						28
3				1																	1	1	3		1	1	2		1		2					11
4	1			1		1																1	1	1					1							7
5																								1												3
6																							1													2
7																																				
8																																				
Totals	1	14	64	243	94	107	59	12	13	1	1	1	6	1	----	1	----	6	1	12	22	20	44	7	16	23	11	----	3	5	3	----	----	----	----	791

In efficie sider greas grow timu appr 18° temp mine Hoff temp as se ston

Dete abs 40° Dete cie to Dete ass ass

TABL date head

Aco tion for c hour

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In calculating the time-temperature efficiency units it is important to consider the effectiveness of varying degrees of temperature in promoting growth. As plants approach the optimum temperature the growth rate is approximately doubled for each rise of 18° in temperature above the basic temperature in conformity with van't Hoff's law of chemical reaction velocity, as set forth by Livingston and Livingston (*13*). The daily number of time-temperature efficiency units was determined from thermograph records during the entire heading period of 1923. From these data the total number of such units required to bring each row from date of first heading to date of last heading were calculated.

TABLE III.—*Time-temperature coefficients (by exponential system) adapted to hourly record on thermograph at Davis, Calif. (Doubled growth rate assumed for each 18° F. (10° C.), and using only temperatures above 40° F.)*

Degrees above 40° F.	Time-temperature units	Degrees above 40° F.	Time-temperature units
1	1.00	28	2.82
2	1.04	29	2.93
3	1.08	30	3.05
4	1.12	31	3.16
5	1.17	32	3.29
6	1.21	33	3.42
7	1.26	34	3.55
8	1.31	35	3.68
9	1.36	36	3.84
10	1.41	37	4.00
11	1.47	38	4.16
12	1.53	39	4.32
13	1.59	40	4.49
14	1.65	41	4.67
15	1.71	42	4.85
16	1.78	43	5.03
17	1.85	44	4.85
18	1.92	45	4.67
19	2.00	46	4.49
20	2.08	47	4.32
21	2.16	48	4.16
22	2.25	49	4.00
23	2.33	50	3.84
24	2.42	51	3.68
25	2.52	52	3.55
26	2.62	53	3.42
27	2.72		

According to the method of calculation used, the value of a unit at 41° for one hour is 1, at 59° it is 2 for each hour, at 77° it is 4 for each hour, and so on to the optimum temperature. The added increments of efficiency, calculated for each degree, are shown in Table III. At temperatures above the optimum the growth rate was assumed to decrease at the same rate at which it previously had increased.

Table IV gives the daily time-temperature efficiency units for the entire heading period from March 26 to May 10.

Table V is presented for purposes of comparison to show the number of time-temperature units, as arbitrarily defined previously, which are required to bring head rows of parent varieties from first heading to complete heading. Sunset apparently requires from 850 to 1,050 such units, while Marquis, coming later in the season at higher temperatures, requires a smaller number. It will be observed in Table VII that the class of greatest frequency of the so-called homozygous rows is found at 901 to 950 time-temperature units in both the early and the late groups.

TABLE IV.—*Daily time-temperature efficiency units from March 26 to May 10, 1923, at University Farm, Davis, Calif.*

Date	Daily time-temperature units	Date	Daily time-temperature units
Mar. 25	62.5	Apr. 19	51.5
26	61.5	20	46.2
27	66.0	21	37.1
28	62.4	22	45.1
29	52.9	23	47.1
30	51.8	24	58.5
31	44.5	25	70.8
Apr. 1	37.3	26	50.0
2	35.6	27	42.0
3	35.0	28	39.2
4	35.5	29	41.7
5	42.3	30	50.1
6	38.5	May 1	49.4
7	39.3	2	48.9
8	42.9	3	65.5
9	38.9	4	53.9
10	49.8	5	58.5
11	46.0	6	64.1
12	53.2	7	63.3
13	60.3	8	70.4
14	58.6	9	74.1
15	64.9	10	60.8
16	39.0	11	66.7
17	34.8	12	72.6
18	44.8		

TABLE V.—*Total time-temperature efficiency units required to bring the recorded numbers of individual head rows of parent varieties from first heading to complete heading*

Parent varieties	Numbers of efficiency units										
	501–550	551–600	601–650	651–700	701–750	751–800	801–850	851–900	901–950	951–1,000	1,001–1,050
Sunset								5	7	6	1
Marquis	1	6	6	6	2		4				

TABLE VI.—*Frequency distribution of F_3 progeny based on time-temperature efficiency units required to bring F_3 rows of early and late segregates from the F_2 from stage of first heading to stage of complete heading*

Dates of first heading in F_2 (1922)	Time-temperature efficiency units																														Totals
	501–550	551–600	601–650	651–700	701–750	751–800	801–850	851–900	901–950	951–1,000	1,001–1,050	1,051–1,100	1,101–1,150	1,151–1,200	1,201–1,250	1,251–1,300	1,301–1,350	1,351–1,400	1,401–1,450	1,451–1,500	1,501–1,550	1,551–1,600	1,601–1,650	1,651–1,700	1,701–1,750	1,751–1,800	1,801–1,850	1,851–1,900	1,901–1,950	1,951–2,000	2,001–2,050	
Apr. 7									1																						1
8																															
9																															
10																						1									1
11																															
12																															
13							1																								1
14								2		1								3		1		1									3
15						1	1	1	1	1	1	1																			7
16					1			1	1	1	1						1														6
17								1	4	6	2	2										1	1								16
18		1						2	10	6	5	3	2					3		1		1			1						35
19					1		2	6	8	9	5	5	2	3	1	2		1	1	5	1	2	1	1							56
20					2		1	9	10	6	4	2	6	3	3	3		3	4	5	3	7			1						72
21							4	2	9	7	7	7	7	1	3	4	7	4	8	19	6	2	2	2						1	102
22						1		3	9	7	6	3	4	2	4	3	8	6	7	15	3	3	1	1			1	1			88
23							1	1	3	4	5	2	4	2	3	4	8	4	6	10	8	5	2	2	1		2	1			77
24					1	1	1	2	4	1	3	1	2	2	2		3	4	5	8	4	6		1		3					52
25									2	2	2	3	2	1	1	1	2	2		3	2	2	2					1	1		29
26									4	2				1	1	1	4	2	4	3	2	2	2	1		1					30
27							1	1	4	1	3	1	1	1	2	1	1	2		4	1	3			1		1				28
28							1			1				2				1		2									1		8
29					1	1	1	2	3	1		1		1	1	1		1	3	1	1										19
30			1	1	1	5	2	4	5	2	1			1		1		1	1			1									27
May 1				2	1	5		2	3	1	1	2	4	3				1													25
2				2		2	5	4	7	2	2	4	3	2					1												34
3				1			2	1	2	4	5	3	3		1							1									23
4				1	2	2	3	1	4	4	4	2	2	1		1							1								28
5					1	3		1			1	1	1	1	1			1													11
6						1	1		1		2	1	1																		7
7							1	1			1																				3
8								1	1																						2
9																															
10																															
Totals		1	1	7	11	22	7	48	96	69	61	44	42	26	23	22	34	36	40	76	31	35	12	8	3	2	4	4	3	2	1	791

TABLE VII.—*Frequency distribution of F_3 head rows based on time-temperature efficiency units required to bring* **each head row** *from the date of first heading to complete heading*

TABLE VII.—*Frequency distribution of F_3 head rows based on time-temperature efficiency units required to bring each head row from the date of first heading to complete heading*

Dates of first heading	Groups of time-temperature efficiency units in degrees F.																													Totals
	501–550	551–600	601–650	651–700	701–750	751–800	801–850	851–900	901–950	951–1,000	1,001–1,050	1,051–1,100	1,101–1,150	1,151–1,200	1,201–1,250	1,251–1,300	1,301–1,350	1,351–1,400	1,401–1,450	1,451–1,500	1,501–1,550	1,551–1,600	1,601–1,650	1,651–1,700	1,701–1,750	1,751–1,800	1,801–1,850	1,851–1,900	1,901–1,950	1,951–2,000	2,001–2,050	
1923																														
Mar. 25						1		3		2		2					1	1	1			3									14
26								9		11	5	1	5	3	1	1	2	3	5	2	3	4	1			1		1			66
27					5		3	8		22	32	21	10	5	12	8		2	13	43	9	12	2	4	2		3				249
28		1				1	2	4		7	2	2	2	6	5		21	11	12	5	8	4	3	2	2	1		1			92
29							2	4		6	1	3	5	1		2	21	9	2	24	4	5	3	1	1		2		2	1	112
30						1	3	2		1	3	1	2		1	6	8	4	2	3	3	6		1			2	1	1		56
31										2				1	1	2	2	1	2		4		2								13
Apr. 1									1	3	2			1	1	2	1					1	2								13
2															1					1											1
3																							1								1
4																															1
5												1		1			1	3													6
6																		1													1
7																															2
8						1						1																			6
9									1	2		2	1																		1
10																1															14
11							4	4	1	3				1		1															26
12						3	4	3	6	1	2	2		2	1	2															21
13					1	3	1	4	2	1	3		2	1	3																47
14			2	5	1	7		2	7	5	6		8	4																	11
15						3		2	3				3																		16
16					2	1	5				4	4																			26
17				1	1	2		3	6	6		7																			11
18								1	4		4	2																			3
19					1		1																								5
20						1		3	6																						3
21							1	1	4																						5
22					1			3	1																						3
23						1	2																								1
24				1																											
25																															
26																															
27																															1
28					1																										
Totals		1	2	7	12	25	27	52	99	72	64	49	38	25	25	24	36	35	39	78	31	35	12	10	3	2	6	4	4	1	1	819

Table VI gives the frequency distribution of F_3 head rows by date of first heading of the F_2 parental segregates and by time-temperature efficiency units required to bring F_3 head rows from date of first heading to complete heading. Figure 4 gives a distribution curve of these data. If the one-factor hypothesis for earliness is correct, there should be an equal number of homozygous and heterozygous rows in the F_3 progeny, according to the symbols AA+2Aa+aa, where A equals the factor for earliness and a that for lateness. The total number of individuals observed in this group was 791. When an arbitrary division at the minimum frequency class of 1,251–1,300 time-temperature efficiency units is made and this class included with the heterozygotes, 481 rows, or 60.70 per cent, were homozygous and 39.30 per cent heterozygous, or a ratio of 1.21 : 0.79, which indicates the expected 1 : 1 ratio. By shifting the division slightly to the left this ratio can be obtained almost exactly.

When the division is made at the 1,251–1,300 class, 21 progeny rows of the late segregates in the F_2, or about 11 per cent, are classed as heterozygous. In addition, certain F_2 plants which began to head about midseason gave progeny which were apparently fully homozygous in the F_3. Also 293, or 48.2 per cent, from a total of 609 plants classed as early in the F_2, fall into the heterozygous class. This is nearly a 1 : 1 ratio, whereas that expected is 2 : 1. The deviation from the expected ratio may indicate that the division between homozygous and heterozygous head rows has been placed too high. Furthermore, it may indicate some shortcoming in the method of calculating time-temperature efficiency units. Another possible cause is the difficulty experienced in determining accurately the end point for heading when taking notes.

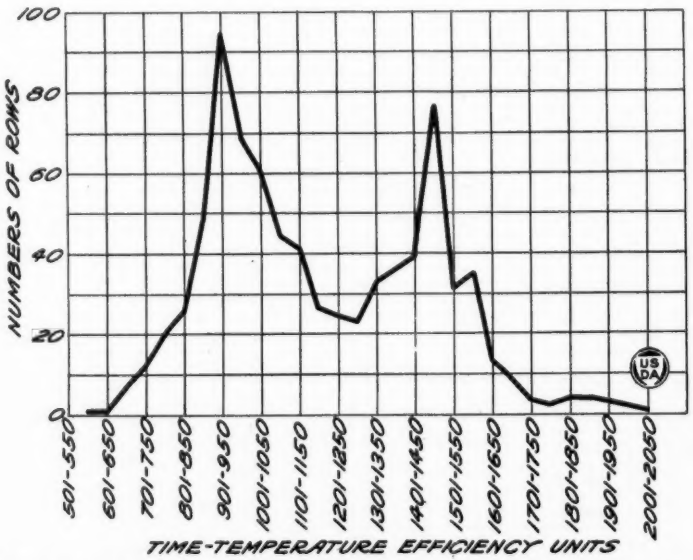

FIG. 4.—Curve showing frequency distribution of F_3 head rows by date of first heading of the F_2 parental segregates and by time-temperature efficiency units required to bring F_3 rows of early and late segregates from the F_2 progeny from first heading to complete heading

Table VII gives the frequency distribution of the F_3 head rows by date of first heading of these rows and by total time-temperature efficiency units required to bring each head row from the date of first heading to the date of complete heading. This table shows a definite segregation into a homozygous late group. Of the total of 819 individuals (including 28 hybrid plants of this cross not tagged for first heading in F_3), 193, or 23.41 per cent, fall into this group. For the whole population this makes a ratio of 3.08 : 0.92, which agrees closely to a 3 : 1 ratio. The total number of individuals in the early group was 627.

TABLE VIII.—*Frequency distribution of the F_3 head-row population with reference to the number of days required for each row to pass from first heading to last heading*

	Number of days																																	Total
	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42				
Number of rows	1	8	10	22	30	30	57	82	69	77	37	22	25	14	21	26	34	47	34	67	38	30	8	10	5	6	4	3	1	1				819

Those classed as early began heading before April 10. Of these, 310 were homozygous and 317 were heterozygous. The minimum frequency class (ordinate 1,251–1,300) was included with heterozygotes. This makes a ratio of 1.00 early to 1.02 late, while the expected ratio is 1 : 2. The reasons assigned above for the discrepancies observed may also apply here.

per row. While the curves from the two sets of data are similar in general outline, a more definite segregation is shown by the latter. The class of greatest frequency in the homozygous group, which developed in the period of low time-temperature units, contains 95 compared with 82 in the same group when the distribution is shown by the days required to develop from first heading to complete heading.

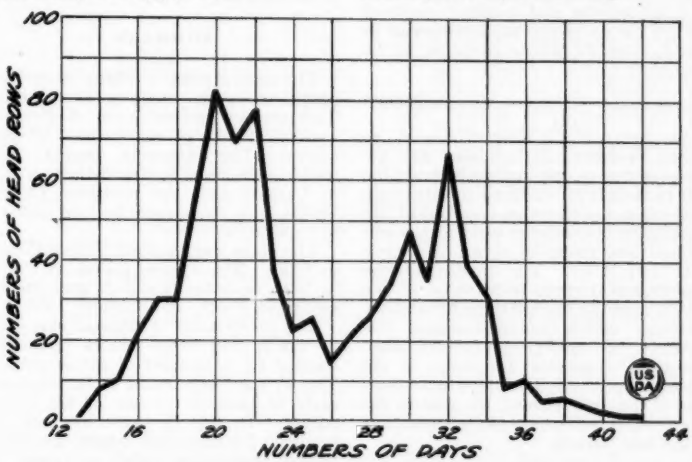

FIG. 5.—Curve showing frequency distribution of the F_3 head-row population based on the number of days required for each row from first heading to last heading

In Table VIII and Figure 5 are shown the distribution of the F_3 head-row population based on the number of days required by each row to develop from the appearance of the first head to the stage when all heads were out. There is shown a large group requiring a comparatively short period and a smaller group requiring a comparatively long period for this development. The division evidently is at 26 days. When half of the individuals of this class were assigned to each group, 58.25 per cent of the rows appeared homozygous and 41.75 per cent appeared heterozygous. This is a ratio of 1.19 to 0.83, which is approximately the same as the segregation on the basis of the total time-temperature units required

Inspection of the data shows that rows requiring the same number of time-temperature units in a number of cases show quite a wide divergence in the total number of days required. This indicates that there was a distinct slowing down of plant growth due to unfavorable temperature conditions, which should be taken into account in a study of this kind. Although a consideration of the time-temperature relation apparently permits a somewhat more accurate analysis of the genetic factors involved in the inheritance of earliness than time alone, it is evident that more information is needed concerning the response of the wheat plant to temperature and other factors which influence growth.

DISCUSSION

EARLINESS IN THE F_1 GENERATION

According to the investigations reviewed earlier in this paper, in two cases (*6*, *3*) the time of maturity in wheat crosses was intermediate in the F_1, while in two others (*2*, *15*) it was inclined to the late parent. In experiments with oats (*4*), corn (*5*), and cotton (*12*), the time of maturity was found to be intermediate; in rice (*10*) it was intermediate but inclined to the early parent; in experiments with peas it was found to be intermediate in one (*1*), while in the other (*10*) it was inclined toward the late parent. The exact behavior in the F_1 was not recorded in the Marquis × Sunset cross reported in this paper. It is very probable that time of maturity is intermediate and possibly inclined toward the early parent, as behavior in the F_2 and F_3 shows earliness to be dominant.

EARLINESS IN THE F_2 GENERATION

The curve of the data for the F_2, as shown by Figure 3, indicates strongly the presence of one main allelomorphic pair of factors for earliness in this cross, as there is a distinct segregation of the population into a large early group and a small late group in a ratio of nearly 3:1 (3.07:0.93). The modes of the two groups diverge from those of the parent varieties toward the intermediate position, which would indicate the presence of modifying factors also. The class of greatest frequency of the early group is only two days later than that of the early parent, whereas in the late group it is five days earlier than that of the late parent.

EARLINESS IN THE F_3 GENERATION

If the inheritance of earliness in this cross can be explained by a single pair of factors, the recessive late group should breed true in the F_3. Nearly 93 per cent of the recessive late individuals from the F_2 were late in the F_3 and, considering the total population, the ratio of early to late was 3.09 to 0.91, which indicates that a one-factor hypothesis for this cross is correct. Further evidence in favor of this view is furnished by the fact that over one-half of the F_2 rows were homozygous for earliness, as shown by the time-temperature unit method of analysis according to segregation in the F_2, $AA+2Aa+aa$.

This experiment is subject to a certain amount of error from various sources. Late or premature heading may be caused by variation in the amount of available soil nutrients, poor drainage, disease, mechanical injury, etc., but with considerable numbers these factors should have little or no effect on results. The index for the end of the heading period is less definite and more difficult to obtain where material is not sacrificed, as was the case in this experiment. Some difficulty was encountered in connection with second-growth culms or tillers which appeared in the late segregates after the spring rains, but these were quite successfully disposed of by ignoring such culms below the knee in height. Close examination was also necessary on account of the large number of culms in each row, yet it is believed that fairly accurate data were procured in this study.

SUMMARY

The cross Sunset × Marquis and its reciprocal were used in this study. The Sunset parent, an Australian variety, is one of the earliest wheats known. The Marquis parent, the well-known hard spring wheat grown in Canada and the northern United States, is a midseason to late variety in California.

The cross was made at Chico, Calif., in 1920. The F_1 was grown at Chico in 1921, and the F_2 and F_3 generations at Davis, Calif., in 1922 and 1923.

The inheritance of time of maturity in wheat and other plants has been studied by a number of investigators. The F_1 has been found to be intermediate or inclined to one or the other parent. In the F_2 there has been a tendency for the intermediate condition to be maintained with a range of variation almost covering the extremes of the parents and both with and without the grouping into classes showing definite segregation.

It is believed that the best index in a study of earliness and lateness in wheat is the date of appearance of the tip of the first spike on the plant. Slight differences are more magnified at this time than at the period of ripening.

No data were secured on the F_1 generation. Other investigators have shown that time of maturity in wheat is either intermediate or inclined to the late parent in this generation.

In the F_2 there was a distinct heaping up of the population into a large early and a small late group in the proportion of 3.07 to 0.93, indicating one allelomorphic pair of factors, with possibly a number of minor modifying factors.

The recessive late group of the F_2 remained late in the F_3, tending to confirm the presence of only one pair of factors for earliness as contrasted with lateness. Further evidence of this view was furnished by the fact that more than one-half of the F_3 population apparently was homozygous for earliness.

These studies indicate that earliness or lateness in wheat is a definitely heritable character, and reports by various investigators show it to vary in complexity in different combinations. Within the common wheat group the character for earliness in winter (habit) × spring (habit) varieties apparently is more complex than in crosses between two spring (habit) varieties.

It is possible to determine progeny rows homozygous for earliness in F_3 and subsequent generations of hybrids by the time required for them to pass through the heading stage. This conclusion is based on the assumption that pure-line rows pass through the heading stage in a time approximately equal to that of pure-line parent varieties.

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THE VITALITY OF BURIED SEEDS[1]

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The distribution and perpetuation of plants depends largely upon seeds. As far back as anything is known of the human race, man has used seeds for plant propagation. Comparatively few species of plants contribute directly to the needs of man, and he has chiefly interested himself in these few. The great remaining flora depends for its reproduction and distribution upon natural conditions. Many plants have effective devices for the scattering of their seed, and often the seeds themselves are safeguarded by a dormant or resting period which prevents them from germinating until the occurrence of favorable seasonal conditions. How long seeds are capable of remaining viable has always been an interesting question, and extravagant statements have frequently been made regarding the germination of seeds of great age. The general impression has prevailed that seeds of various plants are able to retain their vitality for long periods, although buried in the soil. There is in the literature frequent mention of plants resulting from bringing to the top former surface soil which for some reason was buried for a period of years.

Such statements and a desire to obtain accurate data prompted Ewart (*4*)[2] to test for germination many herbarium samples of seed of known age. Many of the seeds which grew were over a hundred years old.

The Gardener's Chronicle in 1894 (*1*) cited a case of a charlock-infested field which was put down to grass and permanent pasture. After 23 years a loaded wagon drawn across the field in the spring when the ground was soft brought subsoil to the surface, and in the following summer a ribbon of charlock grew in the wheel tracks. Peter (*7*) obtained soil samples from various depths in several old forests where the soil had not been disturbed for many years. From these samples he was able to grow seedlings and later to identify the resulting plants. Other similar cases indicate that many seeds can remain alive in the soil, but the length of time is largely speculative.

The first attempt, so far as is known to the writer, to obtain specific data on the length of time seeds are able to retain their vitality while buried in the soil was made by the late Dr. W. J. Beal, of Michigan Agricultural College. In 1879 he buried at East Lansing, Mich., 20 inverted open-mouthed bottles, each bottle containing 50 seeds of each of 20 species. One of these bottles has been taken up every 5 years, the last report (*2*) being made after 40 years, at which time 10 of the 20 species produced sprouts.

Munerati (*6*) planted definite numbers of seeds of several species near the surface of the soil in small squares in an open field which had been kept free from weeds for a number of years. He removed the seedlings as they appeared, keeping a monthly record of the percentage of sprouts. After six years some apparently sound seeds were dug up. Kozma (*5*) buried seeds at varying depths (8, 15, 30, and 50 cm.) in sandy and in loamy soil and recorded the sprouts as they appeared during four and a half years. He compares the effect of loamy and sandy soil on vitality and on the ability of the several species to come up through the different soil depths. In 1902 Dr. J. W. T. Duvel, of the Seed Laboratory, U. S. Department of Agriculture, started the buried seeds experiment, the progress of which this paper describes. He followed the general plan of Dr. Beal but used seeds of a larger number of species and subjected them to more natural conditions.

For the experiment Duvel used 112 samples of seed, representing 107 species. A fixed number of each kind of seed was mixed with sterilized soil taken from the pit where the seeds were to be buried. The mixture of seed and soil was placed in common flower pots. These pots, each covered with a porous saucer, were arranged in sets and buried in the soil at Arlington Experi-

[1] Received for publication June 30, 1924—issued January, 1925
[2] Reference is made by number (italic) to "Literature cited," p. 362.

ment Farm, Rosslyn, Va. As a result of this method, the soil within the pots when taken up has resembled, in moisture and compactness, the soil outside the pots.

In all, 32 sets of pots were buried. The trench in which the pots were buried was approximately 9 feet wide at the top and 50 feet long. On one of its sides the soil was removed to a depth of 8 inches; through the middle the soil was removed to a depth of 22 inches; on the other side it was removed to a depth of 42 inches. Thus the trench had three distinct levels or shelves, each about 3 feet wide, of undisturbed soil. The sets of pots were arranged on each of these shelves, 8 sets on the shallow or A level, 12 sets on the middle or B level and 12 sets on the deep or C level. This arrangement made it possible to take up a set from each of the different depths by digging across the trench. After the pots were removed the earth was immediately replaced to prevent any unnecessary disturbance of the remaining sets. Inasmuch as not more than a foot of soil separated the different sets it was impossible to dig up one set of pots without changing somewhat the temperature, aeration and moisture conditions of the soil surrounding the adjoining set.

The seeds upon being dug up have been tested immediately for germination in the greenhouse. The method employed for making the viability tests has been to sift the contents of each pot on a section of a greenhouse flat nearly filled with sterilized soil. Each flat was divided by partitions into four sections. This arrangement permitted the planting of the same kind of seed from each of the three depths, and a check in the same flat.

A description of the experiment and the results obtained at the end of the first year were published by Duvel (*5*), who continued to supervise the experiment through the test of 1912. The viability tests of 1905, 1908, and 1912 were made by the writer, who since then has continued the experiment. The results of all germination tests are given in Table I.

TABLE I.—*Complete list of seeds buried, with germination obtained each time the seeds were taken up*

Laboratory No.	Burial No.	Name of plant	Number of seeds	Depth	Percentage of germination in—					
					1903	1905	1908	1912	1918	1923
		POACEAE (GRASS FAMILY)								
16173	31	*Agropyron repens* (L.) Beauv. (couch grass).	200	A	20.5	0	0	0	0	0
			200	B	73	1	0	0	0	0
			200	C	66.5	19	0.5	2	0	0
16174	9	*Avena fatua* L. (wild oats)	100	A	9	0	0	0	0	0
			100	B	8	0	0	0	0	0
			100	C	18	0	0	0	0	0
16175	8	*Avena sativa* L. (oats)	100	A	0	0	0	0	0	0
			100	B	0	0	0	0	0	0
			100	C	0	0	0	0	0	0
16176	36	*Bromus secalinus* L. (cheat, chess).	100	A	0	0	0	0	0	0
			100	B	0	0	0	0	0	0
			100	C	0	0	0	0	0	0
16177	37	*Bromus racemosus* L. (upright chess, smooth brome-grass).	200	A	0	0	11.5	0	0	0
			200	B	0	0	42	0	0	0
			200	C	0	0	18.5	0	0	0
16178	66	*Chaetochloa verticillata* (L.) Scribn. (foxtail).	200	A	29	48.5	39.5	30	8	7.0
			200	B	35.5	47.5	37.5	35	3	2.5
			200	C	45	61.5	43	47.5	1	2
16179	33	*Chaetochloa glauca* (L.) Scribn. (yellow foxtail).	200	A	1	2.5	5.5	3.5	0	0
			200	B	1	1.5	1.5	4.5	1.5	0
			200	C	1	2	1.5	1	0	0.5
16180	67	*Chaetochloa viridis* (L.) Scribn. (green foxtail).	200	A	0	55.5	16	38.5	28.5	1.5
			200	B	0	58.5	13	26	5	5.5
			200	C	0	67.5	26.5	79.5	1.5	26
16181	72	*Eleusine indica* (L.) Gaertn. (wire-grass, crab-grass).	200	A	0	3	0.5	0	0	0
			200	B	0	3	1	0	0	0
			200	C	0	2.5	1	0	0	0
16182	15	*Elymus virginicus* L. (Virginia wild rye).	200	A	2	0	0	0	0	0
			200	B	13.5	0	0	0	0	0
			200	C	25.5	0	0	0	0	0
16183	13	*Elymus canadensis* L. (nodding wild rye).	100	A	0	1	0	0	0	0
			100	B	7	1	0	0	0	0
			100	C	22	0	0	0	0	0
16184	14	*Elymus triticoides* Buckl. (wild wheat).	200	A	1.5	0	0	0	0	0
			200	B	3.5	0	0	0	0	0
			200	C	15.5	0	0	0	0	0

TABLE I.—*Complete list of seeds buried, with germination obtained each time the seeds were taken up*—Continued

Laboratory No.	Burial No.	Name of plant	Number of seeds	Depth	Percentage of germination in—					
					1903	1905	1908	1912	1918	1923
POACEAE (GRASS FAMILY)—Continued										
16185	35	*Festuca elatior* L. (meadow fescue).	200	A	0.5	0	0	0	0	0
			200	B	0	0	0	0	0	0
			200	C	0	0	0	0	0	0
16186	12	*Hordeum sativum* Jessen (barley).	100	A	0	0	0	0	0	0
			100	B	0	0	0	0	0	0
			100	C	0	0	0	0	0	0
16187	32	*Panicum virgatum* L. (tall, smooth panicum).	200	A	3.5	7	0	0	0	0
			200	B	8.5	14.5	0	0	0	0
			200	C	8	14.5	0	0	0	0
16188	34	*Phalaris arundinacea* L. (reed canary grass).	200	A	45	39.5	29.5	12.5	12	5
			200	B	46.5	61.5	52.5	46.5	6.5	11.5
			200	C	56.5	62.5	38	15.5	0	1
16189	68	*Phleum pratense* L. (timothy)	200	A	0	0	34.5	22.5	3	0.5
			200	B	0	0	56	22	1.5	5
			200	C	0	0	46.5	51	6	12.5
16190	73	*Poa pratensis* L. (Kentucky bluegrass).	200	A	16	42	0	17	17	13
			200	B	22	65.5	21	4.5	27	10.5
			200	C	24.5	80.5	46.5	3.5	0	18.5
16191	11	*Secale cereale* L. (rye)	100	A	0	0	0	0	0	0
			100	B	0	0	0	0	0	0
			100	C	0	0	0	0	0	0
16192	69	*Sporobolus airoides* Torr. (hairgrass drop-seed).	200	A	0	0	0	0	0	0
			200	B	0	0	6.5	0	0	5
			200	C	0	0	0	8	0	1
16193	71	*Sporobolus cryptandrus* (Torr.) A. Gray (sand drop-seed).	200	A	0.5	0	0.5	0	0	0
			200	B	1.5	0	6.5	0	0	3.5
			200	C	13.5	0	0	0	0	59
16194	70	*Sporobolus cryptandrus* (Torr.) A. Gray (sand drop-seed, hulled seed).	200	A	0	0	0	4.5	0	0
			200	B	0	0	0	7.5	0	20.5
			200	C	0	0	0	0	0	74.5
16195	10	*Triticum aestivum* L. (wheat)	100	A	0	0	0	0	0	0
			100	B	0	0	0	0	0	0
			100	C	0	0	0	0	0	0
16196	1	*Zea mays* L. (corn—Boone County White).	100	A	0	0	0	0	0	0
			100	B	0	0	0	0	0	0
			100	C	0	0	0	0	0	0
16197	2	*Zea mays* L. (sweetcorn—Early Concord).	100	A	0	0	0	0	0	0
			100	B	0	0	0	0	0	0
			100	C	0	0	0	0	0	0
CYPERACEAE (SEDGE FAMILY)										
16198	74	*Cyperus esculentus* L. (yellow nut grass).	200	A	0	1.5	4.5	7	0.5	8.5
			200	B	0	2.5	5.5	21	5	5
			200	C	0	1.5	0	14	0	17
LILIACEAE (LILY FAMILY)										
16199	38	*Allium cepa* L. (onion)	200	A	0	0	0	0	0	0
			200	B	0	0	0	0	0	0
			200	C	0	0	0	0	0	0
CONVALLARIACEAE (LILY-OF-THE-VALLEY FAMILY)										
16200	16	*Asparagus officinalis* L. (asparagus)	100	A	0	0	1	0	0	0
			100	B	0	0	0	0	0	0
			100	C	0	0	0	0	0	0
MORACEAE (MULBERRY FAMILY)										
16201	17	*Cannabis sativa* L. (hemp)	100	A	0	0	0	0	0	0
			100	B	0	0	0	0	0	0
			100	C	0	0	0	0	0	0

TABLE I.—*Complete list of seeds buried, with germination obtained each time the seeds were taken up*—Continued

Laboratory No.	Burial No.	Name of plant	Number of seeds	Depth	Percentage of germination in—					
					1903	1905	1908	1912	1918	1923
URTICACEAE (NETTLE FAMILY)										
16202	75	*Boehmeria nivea* (L.) Gaud. (ramie)	200	A	0	6	0	7	11.0	39
			200	B	0	43.5	0	10	32.0	61
			200	C	0	53.5	0	26	32.0	71
POLYGONACEAE (BUCKWHEAT FAMILY)										
16203	18	(*Fagopyrum fagopyrum* (L.) Karst. (buckwheat).	100	A	0	0	0	0	0	0
			100	B	0	0	0	0	0	0
			100	C	0	0	0	0	0	0
16204	40	*Polygonum pennsylvanicum* L. (smartweed).	200	A	0	0	0	0.5	4.0	0
			200	B	0	0	0	2.5	0	0
			200	C	0	0	1	1	0	0
16205	78	*Polygonum persicaria* L. (lady's-thumb, smartweed).	200	A	0	26	0	60.5	14	1
			200	B	0	1	0	31.5	9	25.5
			200	C	0	0	0	8	.5	55
16206	41	*Polygonum scandens* L. (climbing false buckwheat).	200	A	0	0	0	0	1	0
			200	B	0	0	0	0	0	2.5
			200	C	0	0	0	0	0	5
16207	76	*Rumex salicifolius* Weinm. (willow-leaved dock).	200	A	88.5	67	33	83	57.5	0
			200	B	85.5	75.5	19	62	83.5	72.5
			200	C	70.5	68	23	93.5	89	55
16208	39	*Rumex crispus* L. (curled dock) not cleaned.	200	A	67.5	69	63.5	60.5	42.5	9
			200	B	79.5	64.5	66.5	76	0	24
			200	C	79	58	50.5	72.5	19	13.5
16209	77	*Rumex obtusifolius* L. (broad-leaved dock, bitter dock).	200	A	73	91.5	88.5	82.5	56.5	59
			200	B	72.5	92	84	86	90	77.5
			200	C	79.5	93.5	86.5	89	83.5	82.5
CHENOPODIACEAE (GOOSEFOOT FAMILY)										
16210	81	*Axyris amaranthoides* L. (Russian pigweed).	200	A	0	0	0	0	0	0
			200	B	0	0	0	0	0	0
			200	C	0	0	0	0	0	0
16211	19	*Beta vulgaris* L. (sugar beet)----	100	A	14	0	8	1	0	0
			100	B	39	10	35	1	0	1
			100	C	40	7	9	4	2	1
16212	79	*Chenopodium album* L. (lamb's-quarters, white goosefoot).	200	A	32	36.5	.5	39	30	0
			200	B	63.5	41	50.5	36.5	46.5	0
			200	C	64.5	51	.5	62	47	65.5
16213	80	*Chenopodium hybridum* L. (maple-leaved goosefoot).	200	A	7.5	14	1	36.5	0	0
			200	B	9.5	30	9	27	15	40.5
			200	C	13	34	41.5	39.5	11	0
AMARANTHACEAE (AMARANTH FAMILY)										
16214	82	*Amaranthus retroflexus* L. (rough pigweed).	200	A	9	0	1	11	0	0
			200	B	11	19.5	67.5	36	0	0
			200	C	17.5	12	10	48	0	0
PHYTOLACCACEAE (POKEWEED FAMILY)										
16215	42	*Phytolacca americana* L. (poke, pigeon berry).	200	A	7.5	65.5	29	87.5	0	*0
			200	B	66.5	91	68.5	93	87.5	75
			200	C	80.5	93.5	77	77	93	72.5
PORTULACACEAE (PURSLANE FAMILY)										
16216	83	*Portulaca oleracea* L. (purslane).	200	A	39	15	36.5	21.5	12.5	8.5
			200	B	38.5	30.5	33.5	26.5	20	0
			200	C	30.5	39	11	6.5	14.5	38

* 34 hard seed recovered.

TABLE I.—*Complete list of seeds buried, with germination obtained each time the seeds were taken up*—Continued

Laboratory No.	Burial No.	Name of plant	Number of seeds	Depth	Percentage of germination in—					
					1903	1905	1908	1912	1918	1923
		SILENACEAE (PINK FAMILY)								
16217	43	*Agrostemma githago* L. (corn cockle).	200	A	0	0	0	0	0	0
			200	B	0	0	0	0	0	0
			200	C	0	0	0	0	0	0
16218	84	*Alsine media* L. (common chickweed).	200	A	90.5	64.5	2.5	5.5	0	0
			200	B	96.5	81	47	3	0	0
			200	C	92.5	69.5	50	21.5	0	0
16219	44	*Vaccaria vaccaria* (L.) Britton (cowherb)	100	A	0	0	0	0	0	0
			100	B	4	0	1	0	0	0
			100	C	7	0	1	28	0	0
		BRASSICACEAE (MUSTARD FAMILY)								
16220	87	*Brassica nigra* (L.) Koch (black mustard).	200	A	5	27	0	1.5	0	0
			200	B	7	10.5	1	25	0	38
			200	C	7	9	0	25.5	0	.5
16221	45	*Brassica oleracea* L. (cabbage)	200	A	0	0	1	0	0	0
			200	B	0	0	.5	0	0	0
			200	C	0	0	0	0	0	0
16222	88	*Brassica campestris* L. (turnip)	200	A	0	0	0	0	0	0
			200	B	0	0	1	0	0	0
			200	C	.5	1.5	.5	3	0	0
16223	89	*Bursa bursa-pastoris* (L.) Britton (shepherd's purse).	200	A	0	6	0	2	0	0
			200	B	0	31	4.5	5.5	Missing.	0
			200	C	0	27.5	20	17	47	0
16224	47	*Erysimum cheiranthoides* L. (wormseed, treacle mustard).	200	A	1	0	1	0	0	0
			200	B	2.5	1	0	0	0	0
			200	C	4	0	.5	0	0	0
16225	46	*Neslia paniculata* (L.) Desv. (ball mustard).	200	A	23	3	0	9.5	0	0
			200	B	24.5	5.5	0	8	0	0
			200	C	38.5	10.5	2.5	14	0	0
16226	86	*Sisymbrium altissimum* L. (tall sisymbrium).	200	A	10.5	21.5	79	0	0	0
			200	B	17.5	17	6.5	2.5	0	0
			200	C	26	49	0	17	0	0
16227	85	*Thlaspi arvense* L. (field penny-cress).	200	A	11	9.5	34	0	0	0
			200	B	8	62	52	46	0	.5
			200	C	11.5	31	7.5	10	0	0
		ROSACEAE (ROSE FAMILY)								
16228	90	*Potentilla monspeliensis* L. (rough cinquefoil).	200	A	9.5	89.5	64	20.5	46.5	82.5
			200	B	16	91.5	63	97	Missing.	68.5
			200	C	21.5	95	54.5	88.5	59	91
		CAESALPINIACEAE (SENNA FAMILY)								
16229	48	*Cassia marylandica* L. (wild senna, American senna).	100	A	3	9	14	14	3	1
			100	B	3	23	14	7	5	0
			100	C	5	25	15	14	3	2
		FABACEAE (PEA FAMILY)								
16230	52	*Lespedeza frutescens* (L.) Britton (wand-like bush clover).	200	A	0	.5	0	4.5	0	[b]1
			200	B	0	1	.5	3	0	[c]2
			200	C	.5	1.5	1.5	2.5	0	[d]0
16231	49	*Medicago sativa* L. (alfalfa, lucern).	200	A	2	3.5	.5	0	0	0
			200	B	9	1	.5	0	0	0
			200	C	9	1	0	0	0	0
16232	4	*Phaseolus vulgaris* L. (bean)	100	A	0	0	0	0	0	0
			100	B	0	0	0	0	0	0
			100	C	0	0	0	0	0	0

[b] 15 per cent hard seed recovered. Germinated when clipped.
[c] 32 per cent hard seed recovered. Germinated when clipped.
[d] 48 per cent hard seed recovered. Germinated when clipped.

TABLE I.—*Complete list of seeds buried, with germination obtained each time the seeds were taken up*—Continued

Laboratory No.	Burial No.	Name of plant	Number of seeds	Depth	Percentage of germination in—					
					1903	1905	1908	1912	1918	1923
FABACEAE (PEA FAMILY)—Continued										
16233	5	*Pisum sativum* L. (pea)	100	A	0	0	0	0	0	0
			100	B	0	0	0	0	0	0
			100	C	0	0	0	0	0	0
16234	51	*Robinia pseudacacia* L. (locust tree, false acacia).	200	A	0	5	4.5	21.5	19.5	[e]0
			200	B	0	1	4.5	12.5	6	[f]0
			200	C	0	3	4	18	2.5	[g]0
16235	93	*Trifolium hybridum* L. (alsike clover).	200	A	2	1.5	0	3	2.5	2.5
			200	B	4	3	1	2	.5	4.5
			200	C	4.5	1.5	1.5	5.5	3.5	5
16236	50	*Trifolium pratense* L. (red clover).	200	A	1	0	0	1	.5	3.5
			200	B	2	0	0	.5	.5	3.5
			200	C	2	0	0	2.5	0	[h]1
16237	91	*Trifolium pratense* L. (red clover) harvest, 1900.	200	A	4.5	2	.5	4	4	[h]6
			200	B	5	2	1.5	9.5	Missing.	[h]6
			200	C	6	5.5	1.5	32.5	1	[h]2.5
16238	92	*Trifolium pratense* L. (red clover) hard seed from No. 16237.	200	A	10.5	4	4	1	15	[h]15.5
			200	B	15.5	3	6.5	1.5	Missing.	[h]15.5
			200	C	14.5	4.5	6	3.5	.5	[h]9.5
16239	94	*Trifolium repens* L. (white clover).	200	A	0	0	0	3	2.5	[h]0
			200	B	1	0	0	4.5	0	[h].5
			200	C	0	0	.5	3	1.5	[h]0
16240	3	*Vigna catjang* Walp. (iron cowpea).	100	A	0	0	0	0	0	0
			100	B	1	0	0	0	0	0
			100	C	0	0	0	0	0	0
LINACEAE (FLAX FAMILY)										
16241	53	*Linum usitatissimum* L. (flax, linseed).	200	A	0	0	0	0	0	0
			200	B	0	0	0	0	0	0
			200	C	0	0	0	0	0	0
ANACARDIACEAE (SUMAC FAMILY)										
16242	20	*Rhus glabra* L. (scarlet sumac)	100	A	0	0	0	1	0	0
			100	B	0	0	0	0	0	0
			100	C	2	0	0	0	0	0
MALVACEAE (MALLOW FAMILY)										
16243	54	*Abutilon abutilon* L. (velvet leaf).	200	A	0	0	44.5	69.5	74.5	[i]57
			200	B	0	0	24.5	77	77.5	[h]39
			200	C	0	0	29.5	71.5	76	[i]40.5
16244	6	*Gossypium hirsutum* L. (cotton)	100	A	0	0	0	0	0	0
			100	B	0	0	0	0	0	0
			100	C	0	0	0	0	0	0
16245	55	*Hibiscus militaris* L. (halberd-leaved rose mallow).	200	A	0	0	37.5	74	70	57.5
			200	B	0	0	66.5	80.5	58	36
			200	C	0	0	46.5	74	62.5	46.5
HYPERICACEAE (ST. JOHN'S WORT FAMILY)										
16246	95	*Ascyrum hypericoides* L. (St. Andrew's cross).	200	A	1	0	0	0	0	0
			200	B	0	0	0	0	0	0
			200	C	.5	0	0	0	0	0

[e] 27 per cent hard seed recovered. Germinated when clipped.
[f] 27.5 per cent hard seed recovered. Germinated when clipped.
[g] 31 per cent hard seed recovered. Germinated when clipped.
[h] Some hard seed recovered. Germinated when clipped.
[i] 12.5 per cent hard seed recovered. Germinated when clipped.
[h] 23 per cent hard seed recovered. Germinated when clipped.
[i] 18 per cent hard seed recovered. Germinated when clipped.

TABLE I.—*Complete list of seeds buried, with germination obtained each time the seeds were taken up*—Continued

Laboratory No.	Burial No.	Name of plant	Number of seeds	Depth	Percentage of germination in—					
					1903	1905	1908	1912	1918	1923
ONAGRACEAE (EVENING PRIMROSE FAMILY)										
16247	96	*Onagra biennis* (L.) Scop. (common evening primrose).	200	A	0	48	0	74	57.5	61
			200	B	0	67	0	86	56.5	87.5
			200	C	0	70.5	0	89.5	45	86.5
APIACEAE (CARROT FAMILY)										
16248	57	*Apium graveolens* L. (celery)...	200	A	48.5	18.5	0.5	22.5	1.5	0
			200	B	64	23	3.5	15	1.5	10.5
			200	C	60	38	21	31.5	9	10.5
16249	56	*Pastinaca sativa* L. (parsnip, wild).	200	A	14.5	6	14.5	3	0	0
			200	B	25.5	6.5	11	3	0	0
			200	C	31.5	11	36	13.5	2.5	0
OLEACEAE (OLIVE FAMILY)										
16250	21	*Fraxinus americana* L. (white ash).	25	A	0	0	4	0	0	0
			25	B	0	0	12	0	0	0
			25	C	84	0	4	0	0	0
CONVOLVULACEAE (MORNING-GLORY FAMILY)										
16251	23	*Convolvulus sepium* L. (hedge bindweed, great bindweed).	100	A	2	29	11	41	47	27
			100	B	4	11	14	51	66	41
			100	C	7	13	21	43	64	43
16252	22	*Ipomoea lacunosa* L. (small-flowered white morning-glory).	100	A	20	80	68	22	6	7
			100	B	25	94	72	57	71	52
			100	C	33	88	92	83	2	57
CUSCUTACEAE (DODDER FAMILY)										
16253	98	*Cuscuta polygonorum* Engelm. (smartweed dodder).	200	A	11.5	10	1.5	16	Some.	=25
			200	B	10.5	15.5	4	14.5	Some.	=25
			200	C	13	16	3	9.5	Some.	=25
16254	97	*Cuscuta epilinum* Weihe. (flax dodder).	200	A	15.5	0	0	0	0	0
			200	B	23.5	0	0	0	0	0
			200	C	34	0	0	8.5	0	0
VERBENACEAE (VERVAIN FAMILY)										
16255	100	*Verbena hastata* L. (blue vervain).	200	A	11.5	12.5	0	40.5	0	82.5
			200	B	13	20	0	44.5	0	71
			200	C	14	24	0	26	0	0
16256	99	*Verbena urticifolia* L. (white vervain, nettle-leaved vervain).	200	A	23.5	1	0	19	58	82.5
			200	B	24.5	4	0.5	24	0	90
			200	C	26.5	0	0	20.5	63.5	78
SOLANACEAE (POTATO FAMILY)										
16257	59	*Capsicum annuum* L. (red pepper).	200	A	0	0	0	0	0	0
			200	B	0	0	0	0	0	0
			200	C	0.5	0	0	0	0	0
16258	61	*Datura tatula*, L. (purple stramonium, jimson weed).	200	A	86	41	75.5	77.5	23.5	55
			200	B	84	37.5	98.5	95	82	78
			200	C	86.5	39.5	91	94.5	97.5	55.5
16259	60	*Lycopersicum lycopersicum* (L.) Karst. (tomato).	200	A	0.5	0	0	0	0	0
			200	B	1	0	0	0.5	0	0
			200	C	0.5	0	0	0	0	0
16260	101	*Nicotiana tabacum* L. (tobacco).	200	A	46.5	0.5	28	39.5	19	46
			200	B	70	4.5	45	49.5	25.5	35.5
			200	C	55	18.5	44	78.5	28.5	56
16261	58	*Solanum nigrum* L. (black nightshade, garden nightshade).	200	A	9.5	76.5	31	2.5	0	81.5
			200	B	10.5	66.5	51.5	80.5	19.5	73.5
			200	C	12.5	62.5	27	90	28.5	94.5

= Estimated.

TABLE I.—*Complete list of seeds buried, with germination obtained each time the seeds were taken up*—Continued

Laboratory No.	Burial No.	Name of plant	Number of seeds	Depth	Percentage of germination in—					
					1903	1905	1908	1912	1918	1923
SCROPHULARIACEAE (FIGWORT FAMILY)										
16262	102	*Verbascum thapsus* L. (great mullen).	200	A	7	17.5	11	63	37.5	86
			200	B	7.5	26	7.5	40.5	72	90
			200	C	25.5	29	2.5	82.5	32	92.5
PLANTAGINACEAE (PLANTAIN FAMILY)										
16263	105	*Plantago lanceolata* L. (ribwort, ribgrass, buckhorn).	200	A	20.5	33.5	19	3.5	0	0
			200	B	20.5	50.5	14	2.5	0	0
			200	C	20.5	62	17	4	1.5	0
16264	103	*Plantago major* L. (common plantain).	200	A	39.5	67.5	10.5	52.5	17.5	5.5
			200	B	43.5	59.5	10	32	30.5	13.5
			200	C	46.5	55	14.5	41	19.5	83.5
16265	104	*Plantago rugelii* Dec. (Rugel's plantain, broad plantain).	200	A	12	68	3.5	84	33	0
			200	B	12	55.5	5.5	74.5	29.5	20.5
			200	C	13.5	66	6	16	23.5	37
CUCURBITACEAE (GOURD FAMILY)										
16266	26	*Citrullus citrullus* (L.) Karst. (watermelon).	100	A	0	0	0	0	0	0
			100	B	0	0	0	0	0	0
			100	C	0	0	0	0	0	0
16267	25	*Cucumis melo* L. (muskmelon).	100	A	0	0	0	0	0	0
			100	B	0	0	0	0	0	0
			100	C	0	0	0	0	0	0
16268	24	*Cucumis sativus* L. (cucumber).	100	A	0	0	0	0	0	0
			100	B	1	0	0	0	0	0
			100	C	3	0	0	0	0	0
CICHORIACEAE (CHICORY FAMILY)										
16269	107	*Lactuca scariola* L. (prickly lettuce).	200	A	63.5	74.5	0	0	0	0
			200	B	69	59	0	0	0	0
			200	C	69.5	67	0	0	0	0
16270	62	*Lactuca sativa* L. (lettuce)	200	A	0	0	0	0	0	0
			200	B	0	0	0	0	0	0
			200	C	0	0.5	0.5	0	0	0
16271	106	*Taraxacum erythrospermum* Andrz. (red-seeded dandelion).	200	A	35.5	29	6.5	0	0	0
			200	B	41.5	4.5	0.5	0	0	0
			200	C	45.5	1.5	8	0	0	0
AMBROSIACEAE (RAGWEED FAMILY)										
16272	63	*Ambrosia artemisiaefolia* L. (ragweed).	200	A	16	12.5	12	66	69	2
			200	B	18.5	11.5	6	69	69	83.5
			200	C	20.5	16	3.5	62	81	78.5
16273	28	*Ambrosia trifida* L. (great ragweed).	100	A	0	0	0	5	0	1
			100	B	2	9	0	13	12	15
			100	C	6	8	0	6	2	6
16274	27	*Xanthium pennsylvanicum* Walbr. (cocklebur).	[a] 20	A	0	0	0	5	15	0
			20	B	0	0	45	5	15	0
			20	C	5	0	25	[b]	0	0

[a] Ten fruits buried.

[b] Twenty-five sprouts recorded evidently an error, perhaps due to root sprouting.

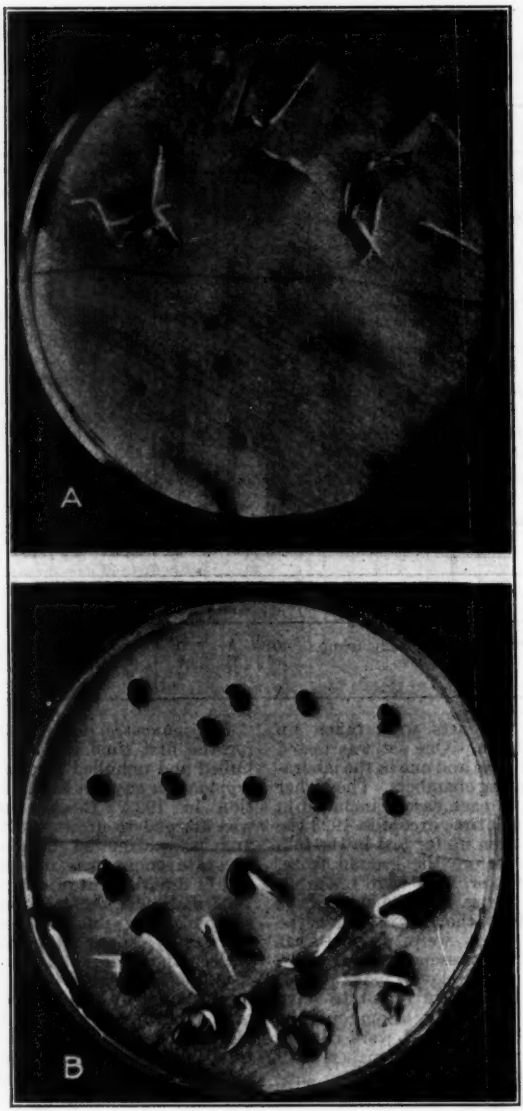
TABLE I.—*Complete list of seeds buried, with germination obtained each time the seeds were taken up*—Continued

Laboratory No.	Burial No.	Name of plant	Number of seeds	Depth	Percentage of germination in—					
					1903	1905	1908	1912	1918	1923
ASTERACEAE (ASTER FAMILY)										
16275	112	*Arctium lappa* L. (burdock, clotbur).	200	A	42.5	31.5	12	53	10.5	0
			200	B	63.5	57.5	16.5	73.5	33.5	29
			200	C	73	54	25.5	93	67	17
16276	64	*Bidens frondosa* L. (black beggar ticks).	200	A	14.5	0	53.	40	0.5	0
			200	B	16.5	0	38	63.5	0	0
			200	C	18	0	60.5	64.5	0	0
16277	111	*Carduus arvensis* L. (Canada thistle).	200	A	21	35	14.5	5.5	3	0.5
			200	B	22.5	28.5	15.5	9.5	0.5	0.5
			200	C	28.5	38.5	25.5	21	3	4.5
16278	110	*Chrysanthemum leucanthemum* L. (whiteweed, oxeye daisy).	200	A	21	78.5	47	78	37.5	42.5
			200	B	33	79	61.5	57	36.5	48
			200	C	49.5	76	81.5	82	20	39
16279	108	*Grindelia squarrosa* (Pursh) Dunal. (broad-leaved gum plant).	200	A	30.5	22	6.5	3.5	0	0
			200	B	36	10.5	1.5	0	0	0
			200	C	42	11.0	5	13.5	0	0
16280	;29	*Helianthus annuus* L. (common sunflower, wild).	200	A	43.5	0	0	0	0	0
			200	B	64	0	0	0	0	0
			200	C	66.5	0	0	0	0	0
16281	7	*Helianthus annuus* L. (common sunflower, cultivated).	200	A	0	0	0	0	0	0
			200	B	0	0	0	0	0	0
			200	C	0	0	0	0	0	0
16282	65	*Onopordon acanthium* L. (cotton thistle, Scotch thistle).	200	A	86	0	82	39.5	36.5	37
			200	B	93	0	90.5	71.5	41.5	4
			200	C	90.5	0	87	61.5	31.5	7.5
16283	109	*Rudbeckia hirta* L. (black-eyed Susan).	200	A	6.5	35.5	1.5	52.5	20	30.5
			200	B	6.5	41	2	57.5	48.5	56.5
			200	C	7	58.5	2.5	66	10	52
PINACEA (PINE FAMILY)										
16284	30	*Pinus virginiana* Mill. (scrub pine, Jersey pine).	100	A	0	0	0	1	0	0
			100	B	0	0	0	0	0	0
			100	C	0	0	0	0	0	0

In 1912, two sets were taken up from each depth. One set was tested in the greenhouse and one in the laboratory germinating chambers. The higher germination in each case is included in Table I. Each time except in 1923 the seeds were taken up for test in the fall, usually just before the ground froze. In 1923, however, they were taken up in the early spring. At the close of the last test (1923) the soil in which the leguminous seeds were planted was examined for the presence of hard seeds. Red clover, white clover, black locust, and bush clover were found to have hard seeds remaining. Some of the hard seeds of each kind were clipped by scratching with emery paper. All so treated sprouted readily when subjected to conditions favorable for germination (Pl. 1).

Polygonum scandens produced sprouts for the first time in 1918. Both the hulled and unhulled seed of *Sporobolus cryptandrus* gave their highest germination in 1923. *Cuscuta polygonorum* was allowed to grow for identification and it became so tangled that an accurate count was impossible. The test of timothy was interrupted in both 1903 and 1905, which accounts for no sprouts being reported for the first two tests, although this seed grew in each of the later tests. In the case of *Xanthium pennsylvanicum* there were no sprouts in 1923, and an examination of the soil in the pots at the time of planting showed only one whole seed from the three depths, the remaining seeds being completely disintegrated.

Robinia pseudacacia produced no sprouts in the flats in 1923. Examina-



Vitality of Buried Seeds Plate 1

A.—*Trifolium pratense*, red clover. Clipped and unclipped seed.
B.—*Robinia pseudacacia*, locust tree. Clipped and unclipped seed.

tion of the soil from the three depths at the expiration of the test showed 27 per cent, 27½ per cent and 31 per cent of hard seed remaining.

For reference a list is appended of the species which produced sprouts in 1923, after being buried in the soil for 20 years. Twelve of these, marked *, have produced sprouts from each depth each year tested. Tobacco is the only cultivated crop included in the twelve.

**Chaetochloa verticillata* (foxtail).
Chaetochloa glauca (yellow foxtail).
Chaetochloa viridis (green foxtail).
Phalaris arundinacea (reed canary grass).
Phleum pratense (timothy).
Poa pratensis (Kentucky bluegrass).
Sporobolus airoides (hair-grass dropseed).
Sporobolus cryptandrus (sand dropseed).
Sporobolus cryptandrus (sand dropseed, hulled seed).
Cyperus esculentus (yellow nut-grass).
Boehmeria nivea (ramie.)
Polygonum persicaria (smartweed).
Polygonum scandens (false buckwheat).
Rumex salicifolius (willow-leaved dock).
Rumex crispus (curled dock).
**Rumex obtusifolius* (broad-leaved dock).
Beta vulgaris (sugar beet).
Chenopodium album (lamb's quarters).
Chenopodium hybridum (maple-leaved goosefoot).
Phytolacca americana (poke).
Portulaca oleracea (purslane).
Brassica nigra (black mustard).
Thlaspi arvense (field penny cress).
Potentilla monspeliensis (rough cinquefoil).
Cassia marylandica (wild senna).
Lespedeza frutescens (bush clover).
Robinia pseudacacia (black locust).
Trifolium hybridum (alsike clover).
Trifolium pratense (red clover).
Trifolium repens (white clover).
Abutilon abutilon (velvet leaf).
Hibiscus militaris (rose mallow).
Onagra biennis (evening primrose).
Apium graveolens (celery).
**Convolvulus sepium* (hedge bindweed).
**Ipomoea lacunosa* (white morning-glory).
Cuscuta polygonorum (smartweed dodder).
Verbena hastata (blue vervain).
Verbena urticifolia (white vervain).
**Datura tatula* (jimson weed).
**Nicotiana tabacum* (tobacco).
Solanum nigrum (black nightshade).
**Verbascum thapsus* (great mullen).
**Plantago major* (common plantain).
Plantago rugelii (broad plantain).
**Ambrosia artemisiaefolia* (ragweed).
Ambrosia trifida (great ragweed).
Arctium lappa (burdock).
**Carduus arvensis* (Canada thistle).
**Chrysanthemum leucanthemum* (oxeye daisy).
Onopordon acanthium (cotton thistle).
**Rudbeckia hirta* (black-eyed Susan).

Typical growth of some plants from the buried seed is shown in Plate 2.

A comparison of the germination of all samples from the different depths shows that the seeds deteriorate somewhat more rapidly at the shallow or A depth. There is little difference between depths B and C. Taking the aggregate of all sprouts obtained in the six tests, 27 per cent came from depth A, 36 per cent from depth B and 37 per cent from depth C.

Of the total number of sprouts obtained in all years from all three depths, the proportion which grew in each year was: 18 per cent in 1903, 19 per cent in 1905, 15 per cent in 1908, 21 per cent in 1912, 12 per cent in 1918 and 15 per cent in 1923.

Table I shows that too much emphasis must not be placed on the actual germination percentage obtained. The results show that under the climatic and field conditions prevailing at Arlington farm, 51 of the 107 species lived 20 years buried in the soil; they do not, however, prove that the 56 species which failed to produce sprouts in 1923 were all dead. Table I shows several cases of growth in 1923 after failure in 1918; and *Polygonum scandens* grew for the first time in 1918. Later tests may bring out similar cases.

The vitality tests have been made as uniformly as possible under conditions which were thought to be best adapted to the majority of the species. However, we know little about the requirements for germination of many species. The variations in the results obtained from the same species in the different years and at different depths for the same year indicate that because of physiological changes in the seeds these requirements will vary with the seasonal and weather conditions prevailing previous to the viability test.

The following 15 species buried in Michigan by Doctor Beal were included by Doctor Duvel in this experiment: *Amaranthus retroflexus, Ambrosia artemisiaefolia, Brassica nigra, Bromus secalinus, Bursa bursa-pastoris, Agrostema githago, Onagra biennis. Plantago major, Portulaca oleracea, Rumex crispus, Chaetochloa glauca, Alsine media, Trifolium repens, Verbascum thapsus* and *Chenopodium al-*

Vitality of Buried Seeds Plate 2

A.—*Carduus arvensis*, Canada thistle.
B.—*Arctium lappa*, burdock.
C.—*Nicotiana tabacum*, tobacco.

bum. With the exception of *Trifolium repens* (white clover), which failed to produce any sprouts in Doctor Beal's experiment and which grew very sparingly every year but one in this experiment, the results check very closely. It might be expected that variations in soil and climate would influence the preservation of vitality of seeds buried in the soil; but too little information is available for comparison and definite conclusions.

Plowing under to exterminate weeds which have gone to seed does not accomplish its purpose. Each plowing of a field infested with weed seeds brings some of these seeds near enough to the surface to germinate, and at the same time buries others deeply enough to preserve their vitality. No normal crop rotation is long enough to effect eradication of persistent weeds.

When the experiment was started, seeds of 35 species were put in paper packages and the packages sealed in glass tubes. These tubes were buried at the deep depth. The seeds in one of these tubes, taken up in 1908, after being buried six years, were tested for germination both in the germinating chamber and in the greenhouse. The results obtained are given in Table II, together with the results obtained the same year from the same species taken from the soil in pots. In most cases the seeds from the tube gave a higher germination than those from the pots. The seeds of the cultivated plants gave distinctly better results from the sealed tubes than from the buried pots.

TABLE II.—*Germination obtained in 1908 in both laboratory and greenhouse from the samples of seeds in the sealed tube buried at depth "C" for six years, together with the percentage of sprouts obtained the same year from the same varieties of seed taken from the soil in pots*

Laboratory No.	Burial No.	Name of plant	Number of seeds	1908 percentage of germination of seeds from—				
				Soil in pots in greenhouse			Sealed tube	
				Depth A	Depth B	Depth C	In laboratory	In greenhouse
16179	33	*Chaetochloa glauca* (L.) Scribn. (yellow foxtail)	100	5.5	1.5	1.5	25	22
16180	67	*Chaetochloa virdis* (L.) Scribn. (green foxtail)	200	16	13	26.5	32	24
16187	32	*Panicum virgatum* L. (tall smooth panicum)	100	0	0	0	35	3
16188	34	*Phalaris arundinacea* L. (reed canary grass)	200	29.5	52.5	38	12.5	0
16192	69	*Sporobolus airoides* Torr. (hair-grass drop-seed)	200	0	6.5	0	69.75	7
16193	71	*Sporobolus cryptandrus* (Torr.) A. Gray (sand drop-seed)	200	0.5	6.5	0	0	5
16204	40	*Polygonum pennsylvanicum* L. (Pennsylvania persicaria)	200	0	0	1	2	0
16207	76	*Rumex salicifolius* Weinm. (willow-leaved dock)	200	33	19	23	73	46
16210	81	*Azyris amaranthoides* L. (Russian pigweed)	200	0	0	0	0.5	0
16212	79	*Chenopodium album* L. (lamb's quarters, white goosefoot)	200	0.5	50.5	0.5	55.5	30
16213	80	*Chenopodium hybridum* L. (maple-leaved goosefoot)	200	1	9	41.5	67	28
16214	82	*Amaranthus retroflexus* L. (rough pigweed)	200	1	67.5	10	94.25	19
16215	42	*Phytolacca americana* L. (poke, pigeon berry)	200	29	68.5	77	86	38
16216	83	*Portulaca oleracea* L. (purslane, pussley)	200	36.5	33.5	11	89.5	9.5
16219	44	*Vaccaria vaccaria* (L.) Britton (cowherb)	200	0	1	1	84	12
16220	87	*Brassica nigra* (L.) Koch (black mustard)	200	0	1	0	0	12.5
16223	89	*Bursa bursa-pastoris* (L.) Britton (shepherd's purse)	200	0	4.5	20	49.25	19
16227	85	*Thlaspi arvense* L. (field pennycress)	200	34	52	7.5	25.5	53
16228	90	*Potentilla monspeliensis* L. (rough cinquefoil)	200	64	63	54.5	52	15
16231	49	*Medicago sativa* L. (alfalfa, lucern)	200	0.5	0.5	0	89.5	73
16235	93	*Trifolium hybridum* L. (alsike clover)	200	0	1	1.5	76.5	55
16236	50	*Trifolium pratense* L. (red clover)	200	0	0	0	85	67
16239	94	*Trifolium repens* L. (white clover)	200	0	0	0.5	84	66
16241	53	*Linum usitatissimum* L. (flax, linseed)	200	0	0	0	90.5	83
16247	96	*Onagra biennis* (L.) Scop. (common evening primrose)	200	0	0	0	22	0.5
16248	57	*Apium graveolens* L. (celery)	200	0.5	3.5	21	72.25	58
16253	98	*Cuscuta polygonorum* Engelm. (smartweed dodder)	200	1.5	4	3	15.25	13
16255	100	*Verbena hastata* L. (blue vervain)	200	0	0	0	0	0
16260	101	*Nicotiana tabacum* L. (tobacco)	200	28	45	44	89.5	43
16264	103	*Plantago major* L. (common plantain)	200	10.5	10	14.5	99	20.5
16265	104	*Plantago rugelii* Dc. (Rugel's plantain, broad plantain)	200	3.5	5.5	6	69.5	0.5
16270	62	*Lactuca sativa* L. (lettuce)	200	0	0	0.5	98	76
16279	108	*Grindelia squarrosa* (Pursh) Dunal., (broad-leaved gum plant)	200	6.5	1.5	5	85.5	36.5
16283	109	*Rudbeckia hirta* L. (black-eyed Susan)	200	1.5	2	2.5	59.25	13.5
16254	97	*Cuscuta epilinum* Weihe. (flax dodder)	200	0	0	0	0	0

SUMMARY

The depth at which the seeds were buried had little effect upon the preservation of their vitality.

Cultivated plants appear to depend largely upon human agencies for their perpetuation.

None of the cereals or legumes whose seeds are used as food germinated on being dug up.

The seeds of weeds or wild plants survived better than those of cultivated plants.

The weed seeds showing the highest germination and the fewest failures were all from common and persistent weeds in the locality of Arlington, Va. The docks, lambs' quarters, plantains, daisies, poke, purslane, jimson, and ragweed are examples.

Of the 107 species buried in 1902, 71 grew in 1903 after 1 year, 61 grew in 1905 after 3 years, 68 grew in 1908 after 6 years, 69 grew in 1912 after 10 years, 50 grew in 1918 after 16 years, and 51 grew in 1923 after 20 years.

The seeds of most weeds, when ploughed under, will not perish during the period of any normal crop rotation.

Any attempt to control weeds which have gone to seed by plowing them under is evidently futile. This conclusion does not invalidate the importance of plowing weeds under before they go to seed.

The preservation of seeds buried in the soil helps to provide a continuous vegetative cover for the land.

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